A Summary of Current Program 7/1/65

and Preliminary Report of Progress

for 7/1/64 to 6/30/65

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EASTERN UTILIZATION RESEARCH AND

DEVELOPMENT DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

and related work of the

STATE AGRICULTURAL EXPERIMENT STATIONS

This progress report is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1964, and June 30, 1965. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Philadelphia, Pennsylvania 19118.

UNITED STATES DEPARTMENT OF AGRICULTURE
Washington, D. C.
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TABLE OF CONTENTS

INTRODUCTION

The mission of the Eastern Utilization Research and Development Division is the development of new and improved products and processes based on designated farm commodities, so as to create new and expanded markets for these commodities. The Division conducts research on dairy products, meat, animal fats, hides, tobacco, maple sirup, honey, and Eastern fruits and vegetables, including potatoes.

In carrying out its mission, the Division does research in physical and biological science and engineering throughout the spectrum of basic research, applied research and pilot plant development. Division scientists are aware of the key role that basic research plays in uncovering new information that may be later exploited in applied research and development. Hence, a substantial portion of the Division's effort is in basic research.

The Eastern Division has a total staff of about 460, including 232 professional research scientists. The Division is organized in 10 laboratories, of which 7 are located at the Eastern Regional Research Laboratory, Wyndmoor, Pa., one is at Beltsville, Md., with part of its research program at Wyndmoor, and two are at Washington, D. C., with one of the latter, the Dairy Products Laboratory, conducting part of its research program at Beltsville. Two of the Division's laboratories are devoted to pioneering research, one in animal proteins and the other research on allergens found in certain agricultural products.

In addition to research in the Division's own laboratories, contract and grant research supported by the Division and equivalent to about 42 professional man-years per year is going forward at 23 locations in the U. S. The Division's program is supplemented by a variety of research projects in foreign countries under P.L.-480 grants.

In every phase of their research, Division scientists cooperate with representatives of colleges and universities, State Experiment Stations, research institutes and associations, industrial organizations and with other government agencies. Much of the cooperation is informal, but some work is conducted under conditions described in written cooperative agreements and memorandums of understanding. Currently 9 such agreements are in effect.

The farm products with which the Eastern Division deals provide more than half of the nation's cash farm receipts. The major part of U. S. farmland suitable for cultivation is used to provide feed for livestock and dairy cattle; in seven states tobacco provides more cash receipts than any other field crop. Hence it is clear that maintaining and enlarging the markets for these and the other farm products under study at the Eastern Division should be a major national concern.

The opportunities are great. A striking illustration is provided by meat and milk; a one percent increase in meat consumption, which might be achieved through improved quality, lower processing costs and new product development, would result in an increase in feed use equivalent to 80 million bushels of corn. Similarly, a one percent increase in milk consumption would result in an increase in feed use equivalent to 25 million bushels of corn.

Division scientists have already achieved much both in terms of discoveries now commercialized and discoveries of a fundamental nature that will be exploited in the future. Some recent examples of progress are as follows:

New Peach Varieties Show Promise for Processing. Several new freestone and clingstone peach varieties with excellent processing characteristics have been discovered as a result of contract research by the New Jersey Agricultural Experiment Station. The results of this research have shown that harvest about three days prior to full maturity yields better canned and frozen products than comparable produce harvested seven days prior to tree ripeness. In each case the fruit was ripened after harvest prior to processing. One variety, which was highest in vitamin A and C contents and outstanding in flavor, will play an important role in future breeding programs. Processors have expressed an interest in propagating some of the most promising varieties selected from these tests. The availability of these superior peach varieties to the canning and freezing industries should lead to increased consumption of processed peaches.

Commercial-scale Gun Designed for Explosive Puffing of Fruits and Vegetables.

Wider commercial development of the process for explosion puffing of fruits and vegetables, should be greatly accelerated by the newly developed puffing gun designed by EU engineers. Detailed construction drawings of the gun have been made available to industry and it is now commercially available. The newly designed puffing gun has an output nearly four-fold that of earlier models. While explosion puffing has been developed commercially on a limited scale, the availability of a commercial-size gun should promote wide-scale adoption of the process, with resulting increased outlets for fruits and vegetables in the processed food industry.

Effect of Storage and Processing Temperatures on Honey Quality Established.

Retaining the good qualities of honey during processing and storage is a special problem in the domestic and export trade. Studies of American honeys show that the rate of quality loss can be predicted accurately from known processing and storage conditions. By lowering storage temperatures 10^{o} to 15^{o} F. quality deterioration can be reduced from about one-fifth to one-third of the rate observed at the higher temperatures. Commercial tests covering normal storage periods have demonstrated that the extra cost of maintaining the recommended lower temperatures is profitably recovered in the increased value of the honey.

Tobacco Utilization Research Reoriented and Expanded. Since publication of the Report to the Surgeon General on Smoking and Health, the emphasis in utilization research on tobacco has been shifted from studies on quality factors to investigations on smoking-health relationships. An extensive program has been initiated on the isolation and identification of new leaf and smoke components which may have physiological effects. Included in this program are studies on the neutral resins of leaf and heterocyclic bases of smoke, which are being conducted under contracts at the Research Triangle Institute, Durham, North Carolina, and the University of Kentucky, respectively. An investigation is underway on the development and evaluation of new cigarette additives capable of altering the smoke composition. The Houdry Process and Chemical Company, specialists in catalysis and process alteration, are under contract for most of this developmental work. An extensive biomedical program will soon be initiated under contract at the University of Kentucky Medical School. This work will consist of providing a biological assaying service for the analysis of experimental tobacco and smoke condensates and of conducting basic studies to develop more rapid biological assaying methods. This reoriented program should play an important role in elucidating the factors responsible for the physiological effects of tobacco smoke.

Genetic Variants of Cow's Milk Caseins Analyzed. Casein, the most abundant protein in milk, is made up of many related but chemically distinct kinds of molecules. Some caseins show heritability characteristics which prove them to be genetic variants of one another. These genetically variant molecules are being analyzed for the numbers of the several amino acids, which are the compositional units that make up the large chainlike molecule of the protein. Multiple substitution of amino acid residues is indicated for some of the variants. Compositional differences among the genetic variants are being related to their facility in forming the molecular aggregates or micelles which contribute the normal whiteness and opacity to milk, and which sometimes aggregate further to form undesirable gels or insoluble sediment. The basic information being acquired in this study may have practical value in accounting for geographic, breed and herd differences in the processing properties of milk. It will be useful in developing means for controlling the aggregation of proteins in processed milks. This work is being done in collaboration with scientists of the Dairy Cattle Research Branch of ARS.

Strontium 90 Removal Process Tested Commercially. Commercial adaptation of the Beltsville fixed-bed cationic ion-exchange method of removing radiostrontium from milk has been achieved successfully through contract research. The milk was processed at a rate of 12,500 pounds per hour in an established commercial dairy which had been equipped with strontium removal equipment. More than 90% of the environmental radiostrontium was removed from the milk without appreciably affecting its appearance, flavor, wholesomeness, or nutritional quality. The additional equipment required can be readily integrated into commercial milk bottling operations without significant increase in processing time. The additional cost for removing radiostrontium from milk is calculated to be from one to two cents per quart, depending on the daily volume of milk being processed and the extent of reuse of removal materials. The method provides a feasible and practical

"standby" means for commercially processing milk in the event of a nuclear emergency.

Composition and Structure of Animal Fats Elucidated. Recent investigations have resulted in the development of unique techniques for determining the composition and structure of animal fats in minute samples of the fat. The procedure involves a hydrolytic breakdown of the fat glyceride by means of enzymes, separation of the hydrolytic products by chromatographic procedures, and conversion of the products to derivatives that permit their analysis by means of thin-layer chromatography. Because of the highly specific action of the enzyme in splitting only certain structures of the fat molecule, this method determines the nature of these structures with absolute certainty. This type of basic information, which is leading to a better understanding of fat composition and structure, is essential to further expansion of animal fat utilization in outlets as fats for improved shortenings and confectionery uses. In addition, because these techniques are particularly adaptable to very small samples, they are now finding wide application in the biomedical field for determining the glyceride composition of fats from tissue of small experimental animals, in studies of lipid metabolism as affected by certain diseases, and in plant genetics where it permits analysis of the fat in a single seed.

Perspiration-resistant Leather. A new tanning process yields leather with increased resistance to deterioration by perspiration, chemicals and washing. The process, based on tanning with glutaraldehyde, is in use by many tanners to produce improved shoe upper, insole, garment, and glove leathers from most types of hides or skins, such as cow, horse, sheep, pig, kangaroo and reptile. The process imparts other desirable properties to leather by increasing its receptivity to dyes, oils, finishes, water-repellent treatments, and other post-tanning operations. These improved properties will place leather in a better competitive position with respect to leather substitutes and should help to preserve this profitable market for animal hides. Estimates indicate that in 1964 over 40,000,000 square feet of leather were produced by the new tanning process. The largest volume use is for work shoes, where perspiration deterioration has always been a problem, but significant amounts are also used for casual and dress shoes.

Dietary Factors in Milk Flavor Investigated. P.L.-480-supported research in Finland is providing new information on the effect of diet on the flavor and quality of cow's milk. Cows maintained on a purified protein-free diet are producing a so-called "zero" milk which serves as a basis for studying the origin of milk flavors. To date, experiments have been concerned mostly with a comparison of milks resulting from protein-free and protein-rich diets. These milks are strikingly similar in their "normal" taste and smell and in their composition. Analysis indicates that even the protein fractions are similar, although slight differences in fat contents and volatile substances do exist and will be investigated further. Extensive studies on the effect of flavor components in certain fodders, in the rumen contents, and in the cow's blood are now underway. Future findings from this research will be of special value to milk processors, who are extremely interested in the origin and

and control of flavor in concentrated and dried milks.

Bacterial Rennets for Cheesemaking Developed. Indian scientists working under a P.L.-480 research grant have reported promising results toward production of bacterial enzymes as substitutes for calf rennet in cheesemaking where its use is restricted by religious beliefs in large areas of the world. Four hundred microbial rennets were prepared and studied for their possible use in cheesemaking. Five emerged as potentially good rennet producers. An additional highlight of this study was the discovery that the phytic acid contained in wheat bran, a cheap agricultural by-product, greatly stimulates enzyme production. It is expected that, as a result of this and related research, bacterial rennets will eventually be produced industrially and that their use will lead to new domestic and export markets for cheese among groups with special food problems and prejudices.

Color Formation in Cured Pork Products Studied. A uniform pink color in cured pork and hams is considered an important quality characteristic in modern meat marketing practices. Research conducted under a P.L.-480 grant in Great Britain is leading to a better understanding of the factors responsible for color irregularities in cured pork products. The research results are assisting to clarify the special roles of cellular constituents and enzyme systems in color production. In the more direct or technological approach, studies with muscle minces are revealing the effect of processing variables upon color formation. One of these findings applicable to commercial curing practices shows that although the initial rate of pigment conversion is slower at lower temperatures, the rate on prolonged incubation may be higher. Continued research along these lines is expected to define the optimum curing environment and to specify those meat enzymes essential to the development of good color in pork products.

It is evident from these examples that the Eastern Division can make highly valuable contributions to agriculture. Indeed, it has been estimated that for utilization research as a whole--adding together the contributions of the Eastern, Northern, Southern and Western Utilization Research and Development Divisions--more than $2.5 billion has been added to the value of products made as a result of the product or process developments of utilization research.

AREA NO. 1 AND AREA NO. 2. DAIRY PRODUCTS - CHEMICAL, PHYSICAL AND BACTERIOLOGICAL CHARACTERISTICS; DEVELOPMENT OF NEW AND IMPROVED PRODUCTS AND PROCESSING METHODS

Problem. Dairying is one of the largest segments of American agriculture: dairy products represent more than 13 percent of all farm cash receipts; milk production requires about 140 billion feed units annually (1 unit is equivalent in feed value to 1 pound of shelled corn); milk is a highly nutritious food. It is clear from these facts that research which succeeds in increasing the consumption of milk will have far-ranging effects in raising nutritional levels, in increasing farmers' income, and in increasing consumption of feeds. There is opportunity to increase milk consumption, for per capita consumption is currently at its lowest point in over 40 years at 585 pounds, whole milk equivalent. Current consumption in the U. S. is well below that of several foreign nations, including New Zealand, Canada, Australia, Sweden, Norway and the United Kingdom, all using more than 800 pounds per capita.

Increased consumption can result from improved quality of manufactured dairy products, from cost reductions based on improved processing technology, from the development of new products, or from any combination of these. The development of new and improved processes and products is the objective of utilization research on dairy products.

Both basic and applied research in this field are needed; applied research is the direct antecedent to the development of new products and processes and basic research provides the information which permits applied research to proceed most effectively.

Increased emphasis on basic research has been advocated by the Utilization Research and Development Advisory Committee and the Animal and Animal Products Research Advisory Committee. Basic research is considered primarily the responsibility of public agencies which disseminate their findings for use by all.

One aspect of the problem posed by dairy products is the great need for fundamental information on the complex biophysical-chemical system which each dairy product is. The development of new products and new processing technology through applied research represents the exploitation of fundamental information. Such exploitation and development cannot continue indefinitely; the supply of fundamental information must be maintained and enlarged, and this is the purpose of basic research. The complexity of milk makes necessary the employment of several scientific disciplines in basic research on this commodity. These disciplines undertake investigations needed to identify and measure the amounts of individual chemical components present. They also seek to define the molecular structure of these components; how the molecules react; and the forces determining the course of the reactions. These studies should be intensified. Other needed investigations

include: study of the synthesis of milk; the properties of milk fat and of the milk proteins; and the factors responsible for the flavor of dairy products and the changes in flavor which occur during processing and storage.

There is also need for a vigorous and sustained program of applied research which is aimed to increase consumption of dairy products. Such a research program could stimulate consumption by development of products with increased palatability, convenience, or extended shelf life. Another opportunity is the possibility of developing new and improved processing technology which will reduce costs. Applied research is most effectively done by technologists closely associated with basic scientists where cross fertilization of ideas can stimulate both groups.

Increased milk consumption, however achieved, should have a powerful upward effect on feed consumption. Since milk production requires about 140 billion feed units annually, a 1 percent increase in milk production would require feed equivalent to 25 million bushels of corn--the production of some 400,000 acres. If the feed were supplied by cropland pasture, almost a million acres would be needed.

It is thus manifest that utilization research on dairy products can provide a powerful stimulus to American agriculture.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving chemists, biochemists, microbiologists, food technologists, and engineers, engaged in basic research on the composition and properties of milk, and in applied research directed to the development of new and improved dairy products and processing technology.

The Department's research facilities are located in Wyndmoor, Pennsylvania, Washington, D. C., and Beltsville, Maryland. The Federal (USDA) scientific effort devoted to research in this area totals 91.5 professional man-years (p.m.-y.), which includes 10.7 p.m.-y. in the domestic contract and grant research program. The effort is distributed as follows:

(a) Work on flavor aspects of dairy products involves 6.0 p.m.-y. at Washington. One category of the flavor studies is identification of desirable flavors and their precursors. Research under a grant (0.8 p.m.-y.) to the University of Maryland, College Park, deals with flavors and flavor precursors in milk derived from pasture or dry feed. Under a grant (1.4 p.m.-y.) to Oregon State University, Corvallis, research is proceeding on the desirable flavor components of butter. A second category of flavor studies is identification and analysis of components responsible for stale flavor. Research under a grant (1.3 p.m.-y.) to Pennsylvania State University, University Park deals with lactones, methyl ketones, and their precursors. In addition, research sponsored by the Department under P.L.-480 grants is in progress at: (1) National Dairy Research Institute, Karnal, Punjab, India, on sulfur compounds in relation to flavor and stability of milk; (2) Biochemical

Institute, Helsinki, Finland, on dietary factors controlling flavor in milk. Research at the Institute of Biochemistry, University of Turku, Finland, on growth-promoting factors for lactic acid bacteria was completed during the year.

(b) Research on whole milk products involves 22.0 p.m.-y. at Washington and Wyndmoor. The program includes fundamental and applied research on development of liquid milk concentrates (6.0 p.m.-y.) and dry whole milk (16.0 p.m.-y.) that will be acceptable to the consumer market in quality and storage stability. In the category of liquid concentrates, a grant to the Ohio State University Research Foundation, Columbus (0.8 p.m.-y.) supports research on the physical state of calcium phosphate-containing casein micelles in the concentrates. A research grant at North Carolina State University, Raleigh, (1.0 p.m.-y.) is concerned with the physical changes associated with steam injection and bubble collapse during milk concentration. Some small-scale consumer testing of dry whole milk is done through the cooperation of the Statistical Reporting Service. In addition to the domestic research on whole milk, the Department sponsors research under P.L.-480 grants at: (1) Technical University Berlin, Berlin-West Germany, on surface-changes in fat globules of dried whole milk; (2) Centro Experimental del Frio, Madrid, Spain, on protein destabilization in frozen concentrated milk; (3) "Juan de la Cierva" Foundation for Applied Research, Madrid, Spain, on thermal and related physical properties of milk and milk products.

(c) Basic research on milk involves 34.6 p.m.-y. at Washington and Wyndmoor. These long-range fundamental studies include the following subjects: structures and interactions of casein and other milk proteins; bacterial spores; structure and properties of nucleic acids; influence of genetics on structure of milk proteins (cooperative with the Animal Husbandry Research Division, ARS);heat stability of milks; milk enzymes; relation of diet of the cow to milk composition. Contract research at the University of Minnesota, St.Paul, (0.7 p.m.-y.) deals with the heat stability problem, and contract research at the University of Maryland, College Park, (0.7 p.m.-y.) deals with the relation of milk fat composition to the diet of the cow. In addition, research sponsored by the Department under P. L.-480 grants is in progress at (1) Indian Institute of Science, Bangalore, India, on phosphoproteins of milk; (2) National Dairy Research Institute, Karnal, Punjab, India, on the proteose-peptone fraction of milk; (3) Institute National de la Recherche Agronomique, Paris, France, on nonprotein nitrogenous constituents of milk; (4) Institute National de la Recherche Agronomique, Paris, France, on proteolytic activity of rennin on casein; (5) Centre de Recherches sur les Macromolecules, Strasbourg, France, on structure of nucleic acids in connection with the synthesis of milk proteins; (6) University of Uppsala, Sweden, on methods for purification of protein complexes applicable to milk; (7) National Institute for Research in Dairying, University of Reading, England, on studies on selected enzymes of milk; (8) Institute Nacional de Tecnologia, Rio de Janeiro, Brazil, on a study of the relation of biological activity of proteins to their structure, as determined by investigations of proteolytic enzymes; and (10) U. P. Agricultural University, Pantnagar, India on dipicolinic acid

synthesis in bacterial spores.

(d) Research on milk fat, cheese and nonfat milk involves a total of 12.0 p.m.-y. in Washington and Beltsville. Study of the production and properties of butteroil involves 4.0 p.m.-y.

Research on improved ripened cheese involves 6.0 p.m.-y.; contract research on the source of Cheddar cheese flavor was completed during the year. Additional research on cheese, sponsored by the Department under P.L.-480 grants is in progress at (1) Kaira District Cooperative Milk Producers Union, Ltd., Anand, India, on potential use of American export nonfat dry milk in manufacture of hard cheese; (2) Institute of Dairy Industry, Warsaw, Poland, on increasing vitamin B content of cheese; (3) College of Agriculture in Olsztyn, Poland, on mechanisms of the cheese-ripening process, and (4) National Dairy Research Institute,Karnal,India,on milk coagulating enzymes. Research on nonfat dry milk involves 2.0 p.m.-y. at Washington. Contract research at the University of Wisconsin, Madison, (0.3 p.m.-y.) is concerned with the effects of nonfat dry milk on bread yeast.

(e) Research at Beltsville on the identification and removal of radionuclides from milk has been discontinued. A research contract with the Producers Creamery Company, Springfield, Missouri, is concerned with development of a commercial scale process for removing radioactive strontium from fluid milk. This contract, supported equally by the Eastern Division and the U. S. Public Health Service, involves a total of 7.3 p.m.-y.

Also, the Eastern Division is supporting jointly with the U. S. Public Health Service contracts with (1) Chemical Separations Corp. on removal of radioactive contamination by use of a moving resin bed; the level of USDA support is 1.9 p.m.-y. and (2) Producers Creamery Company, Springfield, Mo., on removal of radioactive contamination from fluid whole milk by use of a fixed anion-cation resin bed; the level of USDA support is 2.0 p.m.-y.

(f) Pioneering research on the allergens of agricultural products involves 6.2 p.m.-y. at Washington.

PROGRAM OF THE STATE EXPERIMENT STATIONS

The research program on dairy products at the State stations includes studies on basic composition of milk and factors responsible for changes in composition. Some primary concerns are isolation and identification of phospholipids and nonglyceride components of milk fat, influence of feed on fatty acid composition, glyceride structure and physical properties of fat, influence of metal-protein complexes on oxidation of fat, synthesis and structure of fat, formation of solid solutions in mixtures of milk fat, and methods for extracting milk complex lipids.

Another phase of the program deals with the physical and chemical characteristics of fat globule membrane proteins; the physical-chemical properties of kappa-casein and its subfractions; the effects of heat treatment on the

structure, properties and interactions of protein components; the change in shape, size and structure due to enzyme action; the role of casein fractions in curd formation; and the influence of protein fractions on the stability of certain dairy products.

Considerable emphasis is placed on enzyme activity in dairy products. Enzyme studies include the susceptibility of milk to lipolysis; the inhibition of lipases by antibiotics; the number and mode of action of lipases in milk; the influence of lipolytic activity on Cheddar cheese; the chemistry of prorennin activation; the microbial enzymes associated with cheese-ripening; and the characterization of lipases from pseudomonas.

Considerable interest has developed recently regarding residues in dairy products. Research is underway to investigate the effects of pesticides on microorganisms used in cultures; lipolysis of milk fat; activity of purified colostrum pseudocholinesterase; the effects of residues on quality characteristics; and the development of techniques for the detection, removal or neutralization of residue effects.

Research on flavor of dairy products includes identification of compounds in cheese responsible for desirable flavor; chemical nature of off-flavors in milk and dairy products; components responsible for natural flavor of milk; effects of rations on oxidized flavor in milk; off-flavors caused by micro-organisms; effect of minor organic constituents on flavor; and effects of processing treatments on flavor.

The microbiological phases of dairy products research deal with the physiology of mutants of P. roqueforti, with metabolic activity of heat-resistant bacteria, and with factors affecting the growth of bacteria at refrigerator temperatures. Other phases include flavor production of lactic cultures, and growth stimulants and growth retardants of lactic cultures. The incidence of pathogenic organisms in milk and dairy products and the control of these organisms is under investigation. Other studies include factors affecting rate of acid production, bacteriophages active against lactic streptococci, and the isolation of rennet from microorganisms for cheese-making.

Research is underway on developing improved and new processing technology, and improved product quality for dried, concentrated, and frozen products and for butter. Included in this research are the engineering aspects of automation, physical forces involved in circulation cleaning and packaging, including aseptic packaging.

The total number of man-years devoted to this program is 86.5.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Flavor Aspects of Dairy Products.

In the past year attention has been focused on the identification of

desirable flavor components in milk and dairy products. Contract research at Oregon State University, Corvallis, has demonstrated that a trained panel reliably indicates the acceptability of a beverage milk product by the public. To be acceptable the beverage milk should receive a hedonic score of "6.0, like slightly" or higher. This work demonstrates that a trained panel can be a reliable guide to overall consumer acceptability of the products.

Research under a grant to Oregon State University has provided results indicating preference for samples of butter subject to certain manufacturing variables: from sweet cream, cultured cream, cream heated at 160°F. for one hour, and lipase-induced rancid samples. Progress was made in developing techniques for removing the flavor compounds from butter and identifying them.

Good progress was made in studies of the stale flavor of milk products. It is believed that stale flavor is due in major part to the presence of lactones and methyl ketones, and possibly o-aminoacetophenone, in the product. The lactone precursers are a group of hydroxy fatty acid glycerides and a color reagent, the 2,6-dinitrophenylhydrazone of pyruvyl chloride (DNPHPC) was found to react at room temperature quantitatively and quickly with all types of hydroxyl compounds. Methods have been worked out for separating the DNPHPC derivatives of the various classes of hydroxyl compounds; fractionation of the derivatives from milk fat indicates thus far the presence of at least six types of components. Four of these have been tentatively identified as cholesterol, monoglycerides, 1,2-diglycerides and 1,3-diglycerides. Two components not yet identified may be the glycerides containing hydroxylated fatty acids. The o-aminoacetophenone produced from the nonprotein nitrogen under alkaline conditions appears to be influenced by the initial heat treatment of the milk.

In research conducted under a P.L.-480 grant at the National Dairy Research Institute, Karnal, India, a number of alternative and simple methods have been devised for the quantitative estimation of sulfhydryl groups in milk and its products. These methods are proving helpful in identifying the origin and nature of cooked flavors in heat-processed milk.

Research under a P.L.-480 grant at the University of Turku, Finland has yielded much valuable information on the chemical nature of the factors promoting and regulating the growth of lactic acid bacteria isolated from milk products. Some antagonistic relationships between various amino acids were observed in connection with the nutritional requirements of these bacteria. The maximal enzyme activity level was shown to occur in the exponential phase of growth. This information is applicable for commercial enzyme production and provides a guide for obtaining enzymes of optimal activity.

P.L.-480-supported research at the Biochemical Institute, Helsinki, Finland, is providing new information on the effect of diet on the flavor and quality of cow's milk. Cows maintained on a purified protein-free diet are producing a so-called "zero" milk which serves as a basis for studying the origin of milk flavors. To date, experiments have been concerned mostly with a

comparison of milks resulting from protein-free and protein-rich diets. These milks are strikingly similar in their "normal" taste and smell and in their composition. Analysis indicates that even the protein fractions are similar, although slight differences in fat contents and volatile substances do exist and will be investigated further. Extensive studies on the effect of flavor components in certain fodders, in the rumen contents, and in the cow's blood are now underway. Future findings from this research will be of special value to milk processors, who are extremely interested in the origin and control of flavor in concentrated and dried milks.

B. Whole Milk.

1. Vacuum foam-dried whole milk. Good progress has been made on the computer analysis of the main effects of the 13 recognized controllable process variables, that is necessary to determine the optimum operating conditions and to achieve year-round control of foam behavior during drying. The mathematical method being used to interpret and extrapolate the experimental data is based on original work published by the DuPont Company, and a DuPont-developed computer program which precisely fills Division needs was made available to the Division without charge. From the analysis, new process conditions are being obtained from prediction equations derived from experimental data. These conditions will deal with overcoming the seasonal foam variation problem at a moderate though economical dryer product rate. If it is found that year-round process control can be achieved, then data analysis and attendant pilot plant studies will deal with maximizing the product rate while maintaining seasonal control.

2. Foam-sprayed dried whole milk. Concentrated milk foamed by injection of air, nitrogen, or CO_2 before spray-drying yields a product of excellent initial flavor during the fall, winter and spring. However, in summer the initial flavor of the foam-sprayed dried whole milk powder is not of uniform high quality. One possible cause of this difficulty with initial flavor may be the high and variable oxidative capacity of atmospheric air during the warm months.

A spectrophotometric procedure has been devised to measure the oxidative change that takes place in the foam-spray drying operation and to trace the origin of the initial off-flavor that appears in the powder sporadically durint the summer months. This method is capable of accurately determining peroxide numbers as low as 0.001 and can easily determine the amount of oxidation that occurs in one day's exposure. The cellular structure of foam-spray dried whole milk results in a persistent fine-grained foam on reconstitution, especially when the water is cold. An approach to solving this problem has been to meter liquid carbon dioxide into the high pressure milk concentrate feed. This improved the characteristics of the nonfat dry milk but with whole milk concentrates no improvement was noted.

A small-scale consumer-type test of the flavor acceptability was conducted by the Statistical Reporting Service and showed that the average preference for the reconstituted milk was not significantly different from that for fresh whole milk. A companion test revealed a decided preference for fresh nonfat milk over a reconstituted commercial nonfat milk powder. This suggests that the foam-spray dried whole milk powder approximates its fresh milk equivalent more closely than nonfat milk powders approximate the fresh fluid material.

Research on chemical changes occurring at the surface of the fat globules in stored foam-spray dried whole milk at the Technical University Berlin, Berlin, West Germany, under a P.L.-480 grant, has resulted in the development of reliable methodology for investigating the interactions of the carbonyls present in fat with components in the nonfat portion of the powder particle.

3. Liquid milk concentrates. The establishment by Eastern Division research that polyphosphates are the most effective consistency stabilizers known for HTST sterilized concentrated milk represents a major forward step, yet the mode of action of the polyphosphates is unknown. Evidence was found that polyphosphates in amounts up to 0.1% by weight of the finished product are effective antigelation agents in sterile concentrates because of their action in inhibiting secondary changes that occur during sterilization and storage of the concentrates. It is believed that much of the added polyphosphate is converted to pyrophosphate during sterilization, yet addition of the corresponding amount of pyrophosphate to milk either before or after sterilization does not prevent gelation; in fact, it promotes gelation. Still more surprising is the discovery that 0.2% added polyphosphate promotes gelation. A number of sterile milk concentrates containing added polyphosphates are now either being test-marketed or produced commercially in the United States and the evaporated milk industry, in general, is showing great interest in this new and important discovery. Polyphosphates also have a stabilizing effect in frozen milk concentrates and in sweetened condensed milk. In continuing research on the mechanism of gelation, samples of sterilized whole milk were processed in the pilot plant and stored to determine the effect of processing sequence, preheat treatment, and use of polyphosphates on the extent and character of gel formation and sedimentation.

Also, a whole milk concentrate with 44% total solids was produced and remained fluid for over 3 months. With the control of gelation achieved, more attention is now focused on the sedimentation which occurs in HTST sterilized concentrate; it was found that severe agitation of milk during processing is accompanied by greater sedimentation during storage.

In grant research at Ohio State University Research Foundation, Columbus, fundamental data have been obtained on the nuclear magnetic resonance spectra of some casein complexes. Near pH 7 interactions between polypeptide chains in kappa-casein micelles are strong. In grant research at North Carolina State University, Raleigh, investigations have begun on the durability and response time of thermal sensor probes within a steam injector. Results from these two grants are expected to elucidate some of the storage changes in the concentrated milks that should lead to improved processing methods.

Work under a P.L.-480 grant to the Milan Experimental Station for Refrigeration, Italy, led to the discovery that apple and quince pulps added to milk stabilized the milk proteins so that added stabilizers were unnecessary. Apple and quince pulps gave complete stabilization in many combinations with many different fruits. For example, a stable product that tasted like grape juice was obtained by mixing 0.62 parts of grape pulp, 0.4 parts of apple pulp and one part of skim milk. Experiments were carried out on whole milk, skim milk, and powdered milk and with fresh pulp, canned pulp, and frozen pulp. In general, skim milk and fresh pulp were most satisfactory.

A P.L.-480-supported grant with the Centro Experimental del Frio, Madrid, Spain was completed. These investigators report that neither lactose crystallization nor pH change is too significent in protein destabilization during storage of frozen concentrated milk. The final report suggests that the more important factors are the presence of fat, buffer substances and alginates.

C. Basic Research on Milk.

1. Characterization and structure of milk proteins. Solubility studies of two of the genetic variants of β-lactoglobulin, namely A and B, were conducted after the removal of two residues of isoleucine and histidine per molecule from each by means of the enzyme carboxypeptidase A. It was found that the removal of these amino acid residues produced a marked reduction in the solubility of both the A and B forms.

Two treatments with carboxypeptidase were required for the complete removal of two residues of histidine from β-lactoblobulin. After these two treatments, both forms had essentially the same solubility in both water and dilute sodium chloride. Thus, the removal of C-terminal isoleucine and adjacent histidine residues eliminated the differences in solubility of the A and B forms even though the modified A form still contained two more residues of glycine and aspartic acid and two less residues of alanine and valine than did the modified B form, this difference in amino acid composition being of genetic origin. The finding that the large difference in solubility between the A and B forms of β-lactoblobulin is eliminated by the removal of the same four amino acid residues from both forms is unexpected and appears to be inconsistent with the idea that the position or properties of the genetic variant amino acids are responsible for the marked differences in the solubility of genetic variant proteins.

In another investigation the electrophoretic patterns of preparations of the red protein isolated from milk of individual cows suggest the existence of a genetically controlled polymorphism in this protein.

The mechanism of the photooxidation of amino acids in proteins sensitized by methylene blue has been further investigated. The light sensitive amino acids--namely, histidine, methionine, tryptophan and tyrosine--and some of their derivatives, were investigated as a function of pH and temperature. These studies led to the proposal of a cyclic-free radical mechanism for the oxidation of amino acids.

Study of the photooxidation of insulin showed that at 10° only two histidine residues are oxidized and that the rate of oxidation is influenced by the shape of the insulin molecule. A direct correlation was found between the destruction of the two histidine residues in insulin and loss of its biological activity.

Good progress has been made in identifying and characterizing enzymes of milk. A fourth ribonuclease (ribonuclease D) has been found. Milk ribonuclease B is a glycoprotein but no carbohydrate has been detected in ribonuclease A. Milk ribonuclease B differs slightly from pancreatic ribonuclease B.

Good progress has likewise been made in fractionating other milk proteins and characterizing them. The amino acid composition of α_{S1}-A,-B, and C caseins has been completed. Three types of kappa-casein have been found and there is a possibility of a fourth. Since genetic studies on the distribution of the variants of α_{S1}- and β-caseins are now going on throughout the world, it is important that various laboratories agree on the types observed and the use of the techniques for demonstrating these types. For this purpose eight unknown and one standard sample were distributed to the various laboratories and only one sample was wrongly identified by one laboratory. These results indicate agreement on the type identified and satisfactory performance of the methods employed. Fundamental studies underway at the Institute Nacional de Technologia, Rio de Janeiro, Brazil under a P.L.-480 grant have recently provided data which contributes to a better understanding of enzymes which occur in an inactive form and require activation, usually by a proteolytic enzyme before they are functional.

2. Interactions of milk proteins. Study of the solution conformation of milk and other proteins showed that in their native states the three β-lactoglobulins and α_{S1}-casein contain little or no helical structure while α-lactalbumin is nearly 40% helical. Since both α-lactalbumin and β-lactoglobulin have highly folded globular structures, ordered structures other than helical must be predominant in β-lactoglobulin. In nonaqueous solvents major structural changes can take place in the milk proteins, for a high helix content can be induced in the caseins and β-lactoglobulin by dissolving these proteins in organic solvents such as acidic methanol and ethylene glycol. Also, the association properties α_{S1}-casein C/C at pH's between 2-11 show that this protein is strongly aggregated in acid. α_{S1}-casein is dissociated completely by solutions of guanidine hydrochloride into monomeric units of molecular weight 28,400 $\pm$ 2,700. A direct correlation has been found between the proline content and amount of helix formation in a number

of milk and other proteins. This was an expected result because proline is known to interfere with α-helix formation.

In studies of the casein in whole milk concentrates it was found that the effect of hexametaphosphate (HMP) on the caseinate complexes depends upon the concentration of caseinate present. Below a certain critical concentration of caseinate at a given temperature the caseinate is destabilized by HMP. At higher caseinate concentrations the material remains stable for indefinitely long periods at the given temperature. Contract research at the University of Minnesota on the heat-stability of individual milks shows that milk from a cow containing the rare α_{S1}-casein type A/A had very low heat stability.

Investigations of the proteose peptone fraction of milk under a P.L.-480 grant at the National Dairy Research Institute, Karnal, Punjab, India, has resulted in a publication which describes a simple, reliable method for determining the nonprotein nitrogen fraction of both cow's milk and buffalo milk. Studies on selected enzymes of milk under a P.L.-480 grant to the National Institute for Research in Dairying (University of Reading), Shinfield, Reading, England, have resulted in reliable methodology for fractionating lipases present in milk by use of the gel filtration technique. These lipases have been partially characterized in an effort to establish if they are unique to the milk or if they perhaps originated from breakdown of mammary cells.

The research at the Institute Nacional de le Recherche Agronomique, Paris, France, under a P.L.-480 grant on rennin activity and the interactions of milk proteins has produced explanations of some of the phenomena occurring in cheese-making practices. The strict specificity of the clotting activity was confirmed and led to explorations of the exact nature of the chemical bond sensitive to rennin. An important salt effect on rennin activity was revealed. When salt concentration was increased from 0.5% up to 2.5%, rennin activity was considerably reduced.

3. Nucleic acids. Physical-chemical studies on the structural organization of ribosomal RNA (H-RNA) are elucidating the structure of H-RNA from various sources, including rat liver and cow mammary gland. Future work will be carried out with H-RNA samples from cows, bacteria and plants.

Research in progress at the Centre de Recherches sur les Macromolecules, Strasbourg, France, under a P.L.-480 grant on the mechanism of the degradation of DNA by acid deoxyribonuclease has been advanced by the completion of work on the chemical composition of spleen acid deoxyribonuclease. It was demonstrated further that an enzyme from Escherichia coli, endonuclease I, attacks DNA in the same way as acid deoxyribonuclease.

This furnishes another valuable tool in elucidating the role of this enzyme in DNA metabolism and in probing the relationship between genetic factors and the synthesis of proteins.

4. Heat-resistant spores. The cell-free biosynthesis of dipicolinic acid (DPA) by extracts from sporulating cells has been confirmed and purification of the synthesizing enzymes is going forward. Hydrazine is lethal to bacterial spores and makes the spores stainable without release of DPA and hence provides one of the first opportunities to determine the localization of DPA in spores.

D. Milk Fat, Cheese and Nonfat Milk.

1. Milk fat. Results on encapsulation of milk fat as a means of attaining flavor stability have provided capsules containing anhydrous milk fat in edible membranes of gelatin-gum arabic, gelatin-casein, gelatin-sodium alginate, gelatin-carrageenan. Progress has also been made in separating and drying the individual fat-containing capsules.

Contract research on the effect of the diet of the cow on milk composition indicates that cows on lower levels of feed concentrates produce a more highly saturated fat.

2. Cheese. The flavor of low-fat cheese has been improved through the addition of small amounts of γ-lactones and by ripening the cheese milk with bacterial starter an hour or more before setting the curd. The laboratory procedure for making ripened low-fat cheese was scaled up and further tested in the pilot plant at Beltsville. The results of limited informal consumer tests with the cheese were favorable; several hundred persons now have sampled this cheese. Under contract research by the Ohio Agricultural Experiment Station it was found that the intensity of desired flavor in Cheddar cheese is related directly to the concentration of active sulfhydryl (SH) groups formed during ripening. The formation of active SH groups is related to the xanthine oxidase activity, which was greatly reduced by either pasteurization or hydrogen peroxide treatment of the milk. This discovery provides a chemical base on which to develop practical means of improving the kind, intensity and uniformity of cheese flavor. The adverse effect of heating milk or treating it with hydrogen peroxide emphasizes a need for a means of developing flavor in cheese made from pasteurized milk.

Scientists working under a P.L.-480 research grant at the National Dairy Fresearch Institute, Karnal, India, have reported promising results toward production of bacterial enzymes as substitutes for calf rennet in cheesemaking where its use is restricted by religious beliefs in large areas of the world. Four hundred microbial rennets were prepared and studied for their possible use in cheesemaking. Five emerged as potentially good rennet producers. An additional highlight of this study was the discovery that the phytic acid contained in wheat bran, a cheap agricultural byproduct, greatly stimulates enzyme production. It is expected that, as a result of this and related research, bacterial rennets will eventually be produced industrially and that their use will lead to new domestic and export markets for cheese among groups with special food problems and prejudices.

Cheese technologists at the College of Agriculture, Olsztyn, Poland, working under a P.L.-480 grant, have acquired valuable information concerning the chemical changes occurring in Tilsit and Trappist cheeses during ripening. The decomposition of citric acid in Trappist cheese was proved most intensive during the first few days of ripening, continuing slowly thereon for about three weeks more. Electrophoretic and chromatographic studies on protein decomposition during the ripening of Tilsit cheese proved that the enzymatic action of rennet initiated in milk curd continues also in cheese. Protein degradation proceeds on a considerable scale both in the insoluble and soluble fractions of para-casein.

P.L.-480-supported research at the Kaira District Cooperative Milk Producers Union, Ltd. (Anand, India) is showing that the low fat content of native whole buffalo milk can be standardized with reconstituted skim milk for cheese-making purposes. The resulting cheese product has been improved to the point where body breakdown and analytical results are satisfactory, but flavor development is very slow even up to the age of six to nine months.

3. Nonfat dry milk. Equipment capable of concentrating skim or whole milk to as high as 60% solids for foam-spray drying demonstrated the advantages that may be realized by commerical plants upon adaptation of this new drying process, especially for nonfat milk. Foam-sprayed dried nonfat milk can be compressed into packages that would save about 33% in shipping space, without loss of its characteristic instant properties.

Results of contract research at the University of Wisconsin on the use of nonfat dry milk (NDM) in continuous bread-making indicate that NDM can support active fermentation by bread yeast. However, the extent of heat treatment during concentration of the milk solids is important. Yeast activity was increased greatly by NDM from concentrates that were heated at 185°F. for 30 minutes during concentration and further heated at 175°F. for 10 minutes after concentration.

4. Dairy processing equipment. Research has started on the use of liquified gas for foam-spray drying and on the development of a process and equipment for ultra-high concentration of milk. Studies are currently being made on the injection of liquid carbon dioxide and liquid nitrous oxide directly into the high pressure concentrate line feeding the nozzle of the spray drier. The use of the liquified gases would permit substantial economies, improve dispersibility and eliminate surface foaming during reconstitution of nonfat dried milk. The vacuum concentration of milk above 60% solids would provide further substantial economies in milk processing.

E. Radionuclides in Milk.

Under the research contract with Producers Creamery at Springfield, Missouri, it has been shown that 90-95% of the present environmental levels of strontium 90 can be removed from skim and whole milk without appreciably changing the flavor of the pasteurized product. The results indicate that the strontium-90 removal system is operable, efficient, and does not materially change the flavor of milk and that it is commercially feasible to materially reduce strontium 90 levels in milk.

F. Allergens of Agricultural Products.

After several years of intensive search for allergic serum, sera from five individuals allergic to cow's milk have been collected and are being studied to determine the protein fractions of cow's milk responsible for the milk sensitivity. No correlation has been found so far between the allergenic sensitizing capacity of various milk proteins and their previously determined antigenic capacity in experimental animals.

Good progress was made in the study of the antigenic specificity relationships of castor bean meal proteins, castor bean pollen proteins and castor bean allergenic fraction, CB-1A. Guinea pigs could not be sensitized to castor bean pollen antigens by sensitization with CB-1A. CB-1A had less than 0.025% crossed reactive potency with guinea pig sensitized with the pollen. Also, the cross reactive potency of castor bean pollen and castor bean meal antigens was less than 0.4% by the Schultz-Dale test and was 1.6% by a gel diffusion method of testing. CB-1A was separated into three main fractions and the three fractions characterized by disc electrophoresis. Also, CB-1A was fractionated by a semi-preparative method. Specificity relationships of the fractions were determined by gel diffusion analysis and the previous conclusion that chemically different fractions of CB-1A had an identical or common specificity was confirmed. However, a minor specificity was separated from the major specificity and some evidence was obtained for two additional specificities present in trace amounts.

PUBLICATIONS AND PATENTS -- USDA AND COOPERATIVE PROGRAMS

Flavor Aspects of Dairy Products

Dä̈britz, Erich and Virtanen, Artturi I. 1964. S-vinyl-cystein-S-oxyd, vorstufe einer neuen tränentreibenden substanz, vinyl-sulfensäure. Acta Chemica Scandinavica, 18, pp. 837-838.

Honkanen, E., Moisio, T., Virtanen, A. I., and Melera, A. 1964. On the structure of the new amino acid A, 2-methylenecyclopeptene-1,3-diglycine, isolated from the mushroom, Lactarius helvus. Acta Chemica Scandinavica 18, 1319-1320.

Honkanen, Erkki, Moisio, Tauno, Ohno, Minoru, and Hatanaka, Akikazu. 1963. Mass spectra of seven isomeric hexen-1-Ols. Acta Chemica Scandinavica,17, 2051-2054

Kreula, M. and Kresvaara, M. 1965. Isolation of crystalline L-t-vinyl-2-thio-oxazolidone from seeds of cruciferous plants (Cruciferai). Suomen Kemistilehti B38, 1-2.

Matikkala, E. J. and Virtanen, A. I. 1964. Synthesis of 3,3'-(2-methylethylene-1,2-dithio)-dialanine, an amino acid found as γ-glutamyl-peptide in the seeds of chive. Acta Chemica Scandinavica 18, 2009-2010.

Nurmikko, Veikko, Soini, Juhani, Terho, Tuulikki, and Kantonen, Ulla-Maija. 1964. Formation of folate enzymes during the growth cycle of bacteria. II. Activity of serine hydroxymethyltransferase during the lag, acceleration and exponential phases of growth of escherichia coli, streptococcus faecalis, lactobacillus arabinosus and streptococcus thermophilus. Acta Chemica Scandinavica 18, 627-637.

Parks, O. W., Schwartz, D. P., and Keeney, M. 1964. Identification of 0-aminoacetophenone as a flavour compound in stale dry milk. Nature 202, 185-187.

Schwartz, D. P., Keeney, M., and Parks, O. W. 1964. Thin-layer chromatography of carbonyl compounds. Separation of dicarbonyl bis(2,4-dinitrophenylhydrazones) into classes. Microchemical J., 8, 176-179.

Schwartz, D. P. and Parks, O. W. 1963. Thin-layer chromatography of carbonyl compounds. Separation of aliphatic carbonyl 2,4-dinitrophenylhydrazones into classes. Microchemical J., 7, 403-406.

Spåre, C. G. and Virtanen, A. I. 1964. On the occurrence of free selenium-containing amino acids in onion (allium cepa). Acta Chemica Scandinavica 18, 280-282

Virtanen, A. 1964. Versuche über milkproduktion ohne protein under ausschliesscher verwendung von harnstaff und ammonuimstickstaff. Nachrichsen der Gressener Hochschulgesselschaft 33, 73-89.

Virtanen, A. I. and Syvä oja, E. L. 1964. Proteins of cow's milk produced on normal feed and feed containing urea and ammonium salts as the sole nitrogen sources. Suomen Kemistilehti B37, 134.

Virtanen, A. I. and Wahlroos, O. 1963. Absence of 6-methoxybenzoxazolinone in uninjured maize tissue. Journal of Pharmaceutical Science 52, 713-714.

Wahlroos, O. and Saariwista, M. 1964. Benzyl cyanide, benzyl thiocyanate and benzyl isothiocyanate, gas chromatographic data and determination. Acta Chemica Scandinavica 18, 9 2191-2193.

Whole Milk Products

Fox, K. K., Harper, M. K., Holsinger, V. H., and Pallansch, M. J. 1965. Gelation of milk solids by orthophosphate. J. Dairy Sci. 48, 179-185.

Holden, T. F., Aceto, N. C., and Schoppet, E. G. 1964. Effects of viscosity and temperature on the foaming characteristics of concentrated whole milk. J. Dairy Sc. 47, 359-364.

Holsinger, V. H., Fox, K. K., Harper, M. K., and Pallansch, M. J. 1964. Spatial distribution of milk constituents in powders made by different drying techniques. J. Dairy Sci. 47, 964-968.

Komanowsky, M., Sinnamon, H. I., and Aceto, N. C. 1964. Mass transfer in cross-circulation drying of a foam. Ind. Eng. Chem, Process Design Develop. 3, 193-197.

Tamsma, A. and Pallansch, M. J. 1964. Factors related to the storage stability of foam-dried whole milk. IV. Effect of powder moisture content and in-pack oxygen at different storage temperatures. J. Dairy Sci. 47, 970.

Leviton, Abraham, and Pallansch, M. J. January 26, 1965. Process for preparing sterilized milk concentrates. U. S. Patent 3,167,437.

Basic Research on Milk

Alais, C., Blondel-Queroix, J. and Jolles, P. 1964. Etude des substances solubles formées au cours de la réaction de la présure sur la caseine kappa. II. Composition chimique. Fractionnement par l'acid trichloracesique. Bulletin de la Societe de Chimie Biologique XLVI, 973-980.

Alais, C. and Jolles, P. 1964. Action de l'acide acetique et de l'acide trichloroacetique sur la caseine. Ann. Biol. Animale, Biochimie Biophysique 4, 79-90.

Aldridge, W. N., Barman, T. E. and Gutfreund, H. 1964. The rate of formation and decomposition of phosphonyl-phosphatase (Escherichia coli) Biochem. J. 92, 23C-25C.

Bernardi, G. 1964. Chromatography of nucleoside mono- and polyphosphates on hydroxyapatite. Biochim. Biophys. Acta 91, 686-688.

Bernardi, G. 1964. Competitive inhibition of acid deoxyribonuclease by polyribonucleotides. Biochem. & Biophys. Res. Communications 17, 573-577.

Bernardi, G. and Cordonnier, C. 1965. Mechanism of degradation of DNA by endonuclease I from Escherichia Coli. J. Mol. Biol. 11, 141-143.

Bernardi, G. and Griffe, M. 1964. Studies on acid deoxyribonuclease II. Isolation and characterization of spleen-acid desoxyribonuclease. Biochemistry 3, 1419.

Bernardi, G. and Sadron, C. 1964. Degradation of deoxyribonucleic acid by acid deoxyribonuclease (Reprinted from "Acidi nucleici e low furzione biologica"-- Convegno Antonio Baselle, Milan, Sept. 16-18, 1963) Parvia, Fusi 62-80.

Bernardi, G. and Sadron, C. 1964. Studies on acid deoxyribonuclease I. Kinetics of initial degradation of desoxyribonucleic acid by acid desoxyribonuclease. Biochemistry 3, 1411-1418.

Bingham, E. W., and Zittle, C. A. 1964. Ribonuclease of bovine milk: Purification and properties. Arch. Biochem. Biophys., 106, 235-239

Blondel-Quereroix, J. and Alais, Charles. 1964. Étude des substances solubles formées au cours de la réaction de la présure sur la caséin kappa. I. Étude électrophorétique et chromatographique. Bulletin de la Société de Chimie Biologique 46, 963-971.

Disitzer, L. B., Domont, G. B., Iachan, A., and Perrone, J. C. The fractionation of trypsin by electrophoresis in starch columns. Anais da Academia Brasileira de Ciências 36, 43-47.

Domont, G. B., Iachan, A., Disitzer, L. B. and Perrone, J. C. 1964. The amino acid composition of crystalline beef trypsin. Anais da Academia Brasileira de Ciências 36, 137-142.

Downey, W. K. and Andrews, P. 1964. Gel-filtration applied to the study of lipases and other esterases. Biochemical Journal 92, 6-7P.

Dumas, B. Ribadeau, Maubois, J. L., Mocquot, G. and Garnier, J. 1964. Etude de la constitution de la caseine de vache par chromatographie sur colonnes de diethylaminoethylcellulose en milieu uree. Biochimica et Biophysica Acta 82, 494-506*.

Garnier, J., Dumas, B. Ribadeau, and Mocquot, G. 1964. A new method for the preparation of an immunologically homogeneous β-casein. Journal of Dairy Research 31, 131-136*.

Garnier, J., Yon, J. and Mocquot, G. 1964. Contribution a l'etude de l' association entre la casein k et la casein α_s A pH neutre. Biochimica et Biophysica Acta 82, 481-493*.

Gordon, William G. 1964. Macroscopic crystals of bovine α-lactalbumin. Biochim. Biophys. Acta 51, 613.

Gordon, William G., and Basch, Jay J. 1964. Hydrolysis of proteins for amino acid analyses. II. Acid hydrolysis in sealed tubes of mixtures of β-lactoglobulin and starch. J. Assoc. Offic. Agr. Chemists 47, 745-747.

Groves, M. L., Peterson, R. F., and Kiddy, C. A. In press. Polymorphism in the red protein isolated from milk of individual cows. Nature.

Harper, M. K., Curran, H. R. and Pallansch, M. J. 1964. Properties of electrodialyzed bacterial spores. Journal of Bacteriology 88, 1338-1340.

Herskovitz, T. T. 1965. Conformation of proteins and polypeptides. I. Extension of the solvent perturbation technique of difference spectroscopy to study of proteins and polypeptides in organic solvents. J. Biol. Chem. 240, 628-638.

Herskovitz, T. T., Townend, R., and Timasheff, S. N. 1964. Molecular interactions in β-lactoglobulin. IX. Optical rotatory dispersion of the genetic variants in different states of association. J. Am. Chem. Soc., 86, 4445-4452.

Herskovitz, T. T., and Mescanti, L. 1965. Conformation of proteins and polypeptides. II. Optical rotatory dispersion and conformation of milk proteins and other proteins in organic solvents. J. Biol. Chem.. 240. 639-644.

Iachan, A., Domont, G. B., Disitzer, L. B., and Perrone, J. C. 1964. Fractionation of trypsin by paper electrophoresis. Nature 203, 43-45.

Jolles, P., Alais, C., Adam, A., Delfour, A., and Jolles, J. 1964. Recherches préliminaires sur la structure de la partie glucidique des caséino-glycopeptides. Chimia 18, 357-358.

Kalan, E. B., Greenberg, R., Walter, M., and Gordon, W. G. 1964. Chemical properties of β-lactoglobulins A, B and C. Biochem. Biophys. Res. Comm., 16, 199-203.

Kalan, E. B., Thompson, M. P., and Greenberg, R. 1964. End-group analysis of α_{s1}-caseins A,B, and C. Arch. Biochem. Biophys., 107, 521-524.

Kronman, M. J. and Andreotti, R. E. 1964. Inter- and intramolecular interactions of α-lactalbumin. I. The apparent heterogeneity at acid pH. Biochemistry 3, 1145-1151.

Kronman, M. J., Andreotti, R., and Vitols, R. 1964. Inter- and intramolecular interactions of α-lactalbumin. II. Aggregation reactions at acid pH. Biochemistry, 3, 1152-1160.

Kronman, M. J., Cerankowski, L., and Holmes, L. G. 1965. Inter- and intramolecular interactions of α-lactalbumin. III. Spectral changes at acid pH. Biochemistry 4, 518-525.

McMeekin, T. L., and Groves, M. L. 1965. Physical equilibria in milk: Protein. Chapter in "Fundamentals of Dairy Chemistry," edited by B. H. Webb and A. H. Johnson. Avis Publishing Co., Inc., Westport, Conn.

McMeekin, Thomas L., Groves, Merton L., and Hipp, Norbert J. 1964. Refractive indices of amino acids, proteins, and related substances. In Jacob A. Stekol, Symposium Chairman, "Amino Acids and Serum Proteins." pp. 54-66 (Advances in Chemistry Series 44). Washington, D. C., American Chemical Society.

Perrone, J. C., Disitzer, L. B., Domont, G. B., and Iachan, A. 1964. The automatic determination of trypsin activity. Anais da Academie Brasileira de Ciências 36, 131-135.

Susi, H., Timasheff, S. N., and Ard, J. S. 1964. Near infrared investigation of interamide hydrogen bonding in aqueous solution. J. Biol. Chem. 239, 3051-3054.

Thompson, M. P. 1964. Phenotyping of caseins of cow's milk: Collaborative experiment. J. Dairy Sci. 47, 1261-1262.

Thompson, M. P., Kiddy, C. A., Johnston, J. O. and Weinberg, R. 1964. Genetic polymorphism in caseins of cow's milk. II. Confirmation of the genetic control of β-casein variation. J. Dairy Sci. 47, 378-381.

Thompson, M. P. and Kiddy, C. A. 1964. Genetic polymorphism in caseins of cow's milk. III. Isolation and properties of α_{s1}-caseins A,B and C. J. Dairy Sci. 47, 626-632.

Thompson, M. P. and Pepper, L. 1964. Genetic polymorphism in caseins of cow's milk. IV. Isolation and properties of β-caseins A,B and C. J. Dairy Sci. 47, 633-637.

Thompson, M. P., Tarassuk, N. P., Jenness, R., Lillevik, H. A., Ashworth, U. S., and Rose, D. 1965. Nomenclature of the proteins of cow's milk - 2nd revision. J. Dairy Sci. 48, 159-169.

Timasheff, S. N. 1964. The macromolecular parameters of ascites tumor cell H-RNA. Biochim. Biophys. Acta 88, 630-641.

Timasheff, S. N. 1964. Light and small angle X-ray scattering and biological macromolecules. J. Chem. Education, 41, 314-320.

Timasheff, S. N. 1964. The nature of interactions in proteins derived from milk. Published in "Symposium on Foods - Proteins and Their Reactions." Edited by H. W. Schultz and A. F. Anglemier. Published by Avi Publishing Company, Westport, Connecticut. pp. 179-208.

Timasheff, S. N., and Townend, R. 1964. Structure of the β-lactoglobulin tetramer. Nature 203, 517-519.

Townend, R., Herskovitz, T. T., Swaisgood, H. E., and Timasheff, S. N. 1964. The solution properties of β-lactoglobulin C. J. Biol. Chem. 239, 4196-4201.

Townend, R., Basch, J. J., and Kiddy, C. A. 1965. Amino acid analysis of the β-lactoglobulins from individual cows of two phenotypes. Arch. Biochem. Biophys., 109, 325-329.

Treece, J. M., Sheinson, R. S. and McMeekin, T. L. 1964. The solubilities of β-lactoglobulins A,B, and AB. Archives of Biochemistry and Biophysics 108, 99-108.

Weil, L. 1965. On the mechanism of the photooxidation of amino acids sensitized by methylene blue. Arch. Biochem. Biophys. 110, 57.

Woychik, J. H. 1964. Polymorphism in k-casein of cow's milk. Biochem. Biophys. Res. Comm. 16, 267-271.

Zittle, C. A., Kalan, E. B., Walter, M., and King, T. M. 1964. Photooxidation of β-casein. J. Dairy Sci. 47, 1052-1055.

Milk Fat, Cheese and Nonfat Dry Milk

Kristoffersen, T. 1963. Measuring thiamine disulfide reducing substances in Cheddar cheese. Technical Note. J. Dairy Sci. 46, 1135-1136.

Kristoffersen, T., Gould, I. A. and Purvis, G. A. 1964. Cheddar cheese flavor. III. Active sulfhydryl group production during ripening. J. Dairy Sci. 47, 599-603.

Kristoffersen, T. 1964. Effect of purine bases on acid development during Cheddar cheese manufacture. Technical Note. J. Dairy Sci. 47, 806.

Purvis, G. A., Kristoffersen, T., and Harper, W. J. 1963. Formation of sulfur compounds in Cheddar cheese. Abstract. J. Dairy Sci. 46, 602.

Hargrove, R. E., and McDonough, F. E. November 11, 1964. Process of making low-fat ripened skim milk cheese. U. S. Patent 3,156,568.

Radionuclides in Milk

Easterly, D. G., Walter, H. E., and Edmondson, L. F. 1964. Effect of temperature of regenerant on removal of radionuclides from ion-exchange resins. J. Dairy Sci. 47, 1263-1265.

Edmondson, L. F. 1964. Ion-exchange processes for removing radioactive contamination from milk. J. Dairy Sci. 47, 1201-1207.

Allergens of Agricultural Products

Coulson, E. J., and Stevens, Henry, 1964. Ribonuclease of bovine milk: serological relationship to pancreatic ribonuclease. Arch. Biochem. & Biophysics, 107, 336-340.

Coulson, E. J., and Stein, William J. 1965. Passive systemic anaphylaxis in the rat. Federation Proceedings 24, 252.

Spies, J. R., and Coulson, E. J. 1964. The chemistry of allergens. XVI. Ion exchange fractionation of the castor bean allergen, CB-1A, and antigenic specificity relationships of the fractions. J. Biol. Chem. 239, 1818-1827.

AREA NO. 3 - MEATS - PROCESSING AND PRODUCTS

Problem. Livestock production is our greatest single source of farm income. For the past several years over 30 percent of cash receipts from farming were derived from livestock sales. Likewise, the major portion of our land is used to grow livestock feed and forage. Hence, any research which succeeds in stimulating an increase in the consumption of meat and livestock products will have a profound effect on agriculture as a whole. For example, it is estimated that a one percent increase in meat consumption would require an increase in feed equivalent to 80 million bushels of corn.

The processing of meat and meat products also has an important effect on rural industry and rural employment. About half of our meat supply is derived from packing plants in rural areas. Many of these are small (the state of Pennsylvania alone has over 2,000 registered slaughterers) and cannot hope to maintain their own research facilities. They employ local labor, and their products are transported and sold by local truckers and business men. Thus, increases in meat consumption and improvements in meat technology will contribute to increased rural prosperity.

Increases in livestock consumption may be achieved through development of new or improved meat products, or through improved meat processing technology which results in lower costs. In addition, increases in the value of hides, animal fats, and renderers' proteins will benefit the livestock industry by providing additional revenues which could permit reduction in meat prices (thus stimulating consumption) or which could flow back through the marketing channels in whole or in part to livestock growers and feed producers. For example, it is estimated that loss of the market for hides would cause an increase of meat prices that would result in a decrease of 2 percent in meat consumption. Such a decrease would eliminate a market for feed equivalent to 160 million bushels of corn. Conversely, an increase in hide values would operate in the opposite direction and would result in greater income to the livestock industry and in increased utilization of feed grains.

Increased livestock consumption requires both basic and applied research. Applied research is the forerunner of commercial practice and is an indispensable element in successful development. But applied research depends on new knowledge which must be developed in fundamental studies. Our supply of fundamental knowledge must be maintained and expanded if applied research is to be effective and fruitful. This need for basic research has been pointed out by the Animal and Animal Products Research and Marketing Committee, by the Utilization Research Advisory Committee, by the National Agricultural Research Advisory Committee, and by other responsible meat industry groups.

For the reasons given above, research which succeeds in increasing meat consumption can have a powerful effect on American agriculture. The potential effect may be assessed from the facts that meat has a high elasticity demand (a 1 percent drop in retail prices will result in a 0.7 percent increase in

consumption); the production of one pound of livestock requires the equivalent of 7 to 8 pounds of feed grains; and the present United States consumption of meat (174 lb./person in 1964) is still below that of Australia (234 lb.), New Zealand (222 lb.), or Uruguay (234 lb.). Economists predict that the 1965 United States consumption will be lower than in 1964.

Attaining increased meat consumption and providing new technological information for small processors will require a vigorous and balanced research program. There is need for more applied research on processing and preservation, including expanded studies on increasing the efficiency of sausage production and on new dried or semi-dried and ready-to-eat products. Of even greater importance is the need for more basic research on the physical, chemical, and microbiological properties of meat to provide a fund of knowledge for future technological improvements.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving chemists, biochemists, microbiologists, and food technologists engaged in both basic studies and in the application of known principles to the solution of problems in the processing of meat and meat products. The Department's research facilities are located at Beltsville, Maryland, and at Wyndmoor, Pennsylvania.

The Federal scientific effort devoted to research in this area totals 31.4 professional man-years including 6.8 p.m.-y. of contract and grant research. This effort is applied as follows:

(a) Research on microbiology of meat and meat products involves 7.6 p.m.-y. at Beltsville, Maryland. In addition, contract research at Iowa State University (1.6 p.m.-y.) seeks to develop new cured products of distinctive flavor through a study of the fungi associated with cured meat.

(b) Product stability studies involve 4.7 p.m.-y. at Beltsville, Maryland. A grant at Florida State University (1.0 p.m.-y.) is devoted to a study of the relationship between heme pigments and oxidative rancidity in cooked and frozen meats. Another grant at Rutgers University (0.6 p.m.-y.) has been awarded for the study of the non-carbonyl compounds associated with rancid meat. A research contract at the University of Missouri (1.0 p.m.-y.) is devoted to the study of flavor stability and the time-temperature-tolerance of precooked, frozen meat products.

(c) Study of meat composition and quality involves 6.6 p.m.-y. at Wyndmoor, Pennsylvania. A research contract at Louisiana State University (1.4 p.m.-y.) is concerned with the modification of muscle connective tissue constituents and their relationship to tenderness.

(d) Research on meat flavor and smoke composition occupies 5.7 p.m.-y. at Wyndmoor, Pennsylvania. In addition, there is a research contract devoted to the design of new smoked meat products at Michigan State University (1.2 p.m.-y.)

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

For many years State stations have conducted both basic and applied research on problems related to meat utilization. Program scope extends from study of the effects of pre-slaughter treatments on meat quality to development of new or improved meat products.

Pre-slaughter factors such as the effect of breed, diet, stress, environmental conditions and hormonal supplementation are evaluated in terms of the ultimate relationships to quality, color, water binding capacity, structure and composition of meat. Physical and biochemical characteristics of meat are also related to flavor and acceptability of the meat. Such factors as marbling and maturity have long been implicated as factors affecting the utilization of meat. For example, recent results indicate that wide extremes in carcass maturity influence the eating quality of beef a great deal more than wide extremes in marbling.

Among the basic studies related to meat utilization is the continuing program designed to discover the biological mechanism of muscle contraction and to elucidate its relationship to meat tenderness. Study of post-mortem glycolysis in portions of the same pork muscle, which differ markedly in red fiber content provides a unique opportunity to estimate comparative differences in the rate and extent of glycolysis. Effects of post-mortem temperature on pH decline, glycogen depletion, lactic acid accumulation, muscle color and expressible juice are being measured. Other work seeks information on the association of various physiological factors to the post-mortem properties of musculature.

Since the major hydrolytic enzymes of animal tissues are organized within subcellular organelles (lysosomes), the relationship between the chemical composition, structure and function of lysosomes is being determined. Lysosomal enzymes are important in many processes which occur in meats including those of post-mortem autolysis, aging and tenderization and hydrolytic production of flavor constituents. Study of the components of lysosomes has revealed that the lysosomal enzymes include cathepsins, nucleases and glycosidases.

Considerable research effort is devoted to isolation and characterization of meat proteins. Proteolytic changes occur in beef muscle during aging. Muscle proteins are being extracted, fractionated, and their properties used to follow changes occurring during aging. Sarcoplasmic proteins have been fractionated into 18 components. Absorption spectra indicate that these fractions contain several enzymes directly involved in muscle metabolism. Since the major portion of the catheptic activity of beef muscle occurs at pH 4, initial effort will be aimed at purification and characterization of this enzyme. There is need for a rapid, quantitative assay technique for proteolytic enzymes in meat products and further work will be directed along these lines.

The chemical nature of meat pigments continues to be studied. The color stability of freeze-dried meats and problems of pigment fading in cured meats

are of interest. Reflectance spectrophotometry methods are being developed for use in the analysis of pigments at the surface of fresh meat and should help to better understand meat discoloration due to oxidation of myoglobin. The appearance of prepackaged meat is important to consumers who often assume color of meat to be an indication of fresh quality. Research seeks the cause(s) of meat discoloration and means for control of meat color.

As is the case with most foods, investigations of the components of meat flavor are in progress. Workers seek to determine the role of fatty acids, volatiles and amino acids in flavor. Histological studies evaluate structural changes and relate these to eating quality.

Microbiological research is concerned with the microbial aspects of cooking, packaging, storage. curing, fermentation and aging of fresh, prepackaged and processed meat items. Radiation resistance of certain bacteria is being investigated.

An extensive program of work is directed to development of new products and processes. The problems of processing are approached through study of reactions which occur during curing, smoking, aging, freezing, freeze-drying and prefabrication. Product stability studies are also in progress.

A regional project, WM-33, covers a portion of the meats research reported.

The total research effort on meat is carried out at 41 stations and involves approximately 60.6 p.m.-y.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Microbiology of Meat and Meat Products.

The mechanism by which some microorganisms can grow at low temperatures, the so-called "psychrophiles," is being investigated with a strain of Escherichia coli through radioactive tracer studies of the interrelationships of cell yield, temperature and substrate utilization. Present data indicate that more energy is required to maintain or produce a given level of cells at low temperatures than at higher temperatures. Additional studies in this area should indicate the metabolic pathways involved.

Continuing studies on microbial activity on fats have shown that the lipase from a psychrophile, Pseudomonas fragi,is truly extracellular. Disc electrophoresis has shown two bands of lipolytic activity instead of the single activity previously noted, and purification by density gradients on Sephadex columns has eliminated a proteinase contaminant. Studies on specificity with this enzyme should now be more reliable. The lipase previously isolated from Geotrichum candidum has been shown to be stereo-specific for the cis-form of oleic acid. This is not true with pancreatic, milk, or wheat germ lipases. This specificity may prove useful in the study of the triglyceride structure of natural fats and oils.

Investigation of the role of microorganisms in non-hydrolytic rancidity in fats has shown that a number of microorganisms actually utilize carbonyls and peroxides. It also has been found that the activity of some microbial lipases is severely limited by certain unsaturated fatty acids.

Food poisoning caused by Clostridium perfringens occurs rather frequently in Europe and sporadically in the United States. Although it is usually associated with meat and meat products, it is rarely related to cured meats. Studies of the past year on the effect of curing salts and smoking on the growth and survival of this sporeformer in cured meats and hams have shown that the organism will survive the entire process, even when present at a low level of contamination. Thus, sanitation rather than an inhibitory curing process probably accounts for the low incidence of outbreaks from cured meats.

Staphylococci are the most common cause of food poisoning outbreaks from meats. A method recently developed by the Food and Drug Administration for the in vitro measurement of the staphylococcus enterotoxin is being used to study the effect of curing agents and temperature on toxin production.

In the contract project at Iowa State University on the relationship of fungi to the development of flavor, numerous fungal cultures have been isolated from hams exhibiting desirable flavor. Studies are now underway in which hams are being inoculated with these cultures to determine their effect on flavor under controlled conditions.

A P.L.-480 project has recently been negotiated with the Central Institute for Nutrition Research in Zeist, to investigate the effect of enterotoxins of microorganisms on protozoa. The aim of this research is to develop a sensitive assay system that will permit the rapid estimating of microbial enterotoxins in foods.

B. Product Stability Studies.

The isolation of free carbonyl compounds in the determination of flavor changes and deterioration in meat and meat products is important because of the major effect such compounds exert. In lipid oxidation, potential carbonyl compounds may be enormous compared with the free carbonyls, and the precursors and capacity for change complex and sensitive. Comparisons of quantitative methods for isolation of aldehydes showed wide differences in amounts separated from oxidized lard. The Girard T method, which was developed in this research, came closest to a quantitative isolation of free aldehydes. In this work a new, highly unsaturated class of aldehydes was isolated.

Studies have been made on relationships of specific aldehydes to rancid flavors. Judges were able to describe the presence of individual aldehydes of various classes in amounts ranging from 2 to 15 ppm. The contribution of an aldehyde to rancidity became greater with increasing chain length up to C_{10}. The volatile aldehydes of a rancid lard contained individual aldehydes totaling only 1.1 ppm. This may indicate a powerful additive effect or the influence of other compounds. The characteristic aroma and flavor of lard

disappeared prior to the detection of off-flavors due to oxidation.

Preliminary experiments suggested that differences in stability of pork, beef, and lamb fat might not be due entirely to composition variation but to other factors such as glyceride structure. Back and leaf fat from lines of lard- and meat-type hogs have been processed for study of related variations in ketoglycerides, composition, and stability.

Under the grant at Florida State University, new methods have been developed for the determination of total meat pigments, percentage of metmyoglobin, and the enzymatic reduction of metmyoglobin. Enzymatic pathways in meat for the reduction of atmospheric oxygen and metmyoglobin are being studied.

In studies under the contract at the University of Missouri, on progressive changes in frozen and precooked frozen meats related to flavor stability, early results showed that heating beef caused increases in adenylic acid and decreases in inosinic acid. Nucleotides, other than adenylic acid, increased during storage at 0°F. Quick thawing of meats stored at 0°F. lowered the content of reducing sugars.

Under the P.L.-480 grant at the University of Helsinki, changes in lipid composition of dry sausages during ripening are under study. These are sausages produced by fermentation and, although similar to dry sausages, are produced in a shorter time. Early results show that oleic acid content decreases during the ripening period. This may be due to changes in glyceride structure or microbial effects. Current work is devoted to studying the lipolytic bacteria found in the sausages and to further investigations of the nature of the lipids present and the manner of their breakdown.

C. Meat Composition and Quality Research.

Investigations of meat animal muscular structure were designed to ultimately lead to improved and enhanced tenderness and juiciness. Myosin and actomyosin, the principal proteins, were isolated, purified, and characterized; and facts pertinent to meat protein analyses were established. Related work, conducted by contract at Louisiana State University, dealt with the histochemistry and biochemistry of connective tissue. Tentative evidence for a relation of specific protein fractions to tenderness was established, and methods were developed for analyses of mucopolysaccharides and mast cell counts.

The research aimed at the improvement and enhancement of color in cured meats involved the isolation of pigments (myoglobins) from beef, lamb, and pork and investigation of their reactivity in meat-curing. Evidence obtained of the relative instability of pork nitrimetmyoglobin may lead to a better understanding of problems in pork-curing. The heat denaturation of the protein components of cured pork was investigated to develop a method for accurately determining the temperature to which meat products have been heat-processed; such a method would have special applications by the Meat Inspection and Animal Inspection and Quarantine Divisions, as well as by processors. Three proteins were isolated having heat denaturation rates that should be useful in

designing the accurate method needed.

Investigations of the manufacture of emulsion-based sausage, such as frankfurters and bologna, were initiated with special attention to accelerating the process through a better understanding of the effect of increasing temperatures above those conventionally used. Changes detected in proteins are being correlated with processing temperatures.

In a P.L.-480 project at the Low Temperature Research Station in England, Dr. S. M. Partridge has investigated the fundamental chemistry of some of the connective tissue proteins. Dr. Partridge isolated and described the structure of a chondroitin sulphate protein complex of bovine cartilage. This is an interesting compound which consists of a protein core surrounded by long, straight, polysaccharide chains. Another compound of connective tissue isolated and described was a sialomucoprotein. Dr. Partride also studied the effect of proteolytic enzymes on these compounds and showed how they were hydrolyzed.

The natural reducing substances in pork tissue which may contribute to the color fixation reaction in meat-curing are being studied under a P.L.-480 grant by Dr. A. McM. Taylor and his associates at the British Food Manufacturers Research Association at Leatherhead, England. The work so far has been concerned with the isolation of pork muscle mitochondria and a study of the enzyme kinetics of the reactions between myoglobin and nitrite. Results indicate that normal cellular metabolic pathways are capable of reducing nitrite to provide nitric oxide under the anaerobic conditions obtaining during curing. The role of mitochondria, mitochondrial enzymes, and other cell constituents is being studied.

In a new P.L.-480 project at Chung Hsing University in Taiwan, Professor Chen You Kong is investigating the production of new types of meat products by a technique similar to drying. Fat is an excellent heat transfer medium for preparing meat products, and our war-time experience with meat dehydration leads us to feel that novel fried meat items may be developed through this research.

D. Meat Flavor and Smoke Composition Research.

The aroma of raw meat, while distinctive, is not particularly attractive for human consumption. After proper processing (cooking, grilling, broiling), a desirable odor develops. Precursors of the odor are present in the meat and, through a series of chemical reactions, give rise to the volatile components associated with the meat aroma. Since the aroma precursors are water-soluble, they are extracted from ground, raw meat. The proteins and large molecular weight material are removed by dialysis; the aroma is associated with the small molecules. The extract is separated by ion exchange fractionation on various resins. A fraction containing amino acids and hypoxanthine has been obtained which has a distinct meaty aroma. Sixteen amino acids, including the dipeptides anserine and carnosine, have been determined quantitatively in this fraction. The removal of hypoxanthine

results in alteration in the aroma. A number of tests, including the specific glucose oxidase assay for glucose, and gas-liquid chromatography of the tri-methyl silyl derivatives, indicate the absence of sugars in the fraction with a meaty aroma. While studies are underway to further confirm this finding, it is of interest because it has been assumed that meat flavor and aroma are due to a "browning reaction" involving amino acids and sugars.

More than 200 compounds are known to be formed by the thermal degradation of wood as used in the preservation of meat by smoking. Identification of the desirable components in good smoke will lead to improved techniques for their selective production. A laboratory scale smoke generator, heated by electricity, has been constructed in such a way that the rate of heating and the air flow can be controlled, temperatures recorded at various points in the system, and samples of smoke obtained in appropriate traps. Analysis of the trapped smoke solution by the use of the gas-liquid chromatograph and infrared spectroscopy has led to the identification of a number of phenols and neutral compounds. While all the components have not yet been identified, the appearance of components as separated by gas-liquid chromatography permits tentative comparisons among smoke preparations. Commercial liquid smoke preparations are being introduced and are being well accepted by the industry. The solutions vary considerably in odor and taste, and gas-liquid chromatography examination shows marked differences in composition between the natural and artificial, or imitation, smoke solutions. Recent literature reports have indicated the possibility of the presence of carcinogens in smoke and smoke solutions, and work is being planned to investigate the significance of these compounds.

Research under the contract at Michigan State University has already developed a smoked ham base which is now being incorporated into commercially feasible products. Other products are in the process of development.

A P.L.-480 grant by Dr. J. Tilgner at the Technical University at Gdansk, Poland, is devoted to studying the antioxidants of smoke. To date, smoke fractions have been isolated, and their antioxidant properties are under study.

PUBLICATIONS AND PATENTS -- USDA AND COOPERATIVE RESEARCH

Microbiology of Meat and Meat Products

Alford, J. A., Pierce, D. A. and Suggs, F. G. 1964. Activity of microbial lipases on natural fats and synthetic triglycerides. J. Lipid Research 5, 390.

McLean, R. A. 1964. Micrococcus cryophilus is not flagellated. J. Bacteriol. 87, 1258.

Product Stability

Eser, H., and Niinivaara, F. P. 1964. Gaschromatographische untersuchungen über das aroma von rohwurst. Sonderabdruck aus "Zeitschrift für Lebensmittel-Untersuchung und -Forschung' 124, 245-251.

Gaddis, A. M., Sulzbacher, W. L., Crowe, P. F. and Suggs. F. G. 1964. Aging of farm-cured hams as affected by method of cutting, curing, and smoking. Util. Res. Report No. 4.

Niinivaara, F. P., Pohja, M. S., and Komulainen, S. E. 1964. Some aspects about using bacterial pure cultures in the manufacture of fermented sausages.. Food Technol. 18, 25.

Pohja, M. S., and Niinivaara, F. P. 1964. Eigenschaften und bedeutung der in rohwurst und in pökellake vorkommenden fett abbauenden bakterien. Die Fleischwirtschaft 44, 435-439.

Stewart, M., Zipser, M. W. and Watts, B. M. 1965. The use of reflectance spectrophotometry for the assay of raw meat pigments. J. Food sci. 30, 464.

Stewart, M. R., Hutchins, B. K., Zipser, M. W. and Watts. B. M. 1965. Enzymatic reduction of metmyoglobin by ground beef. J. Food Sci. 30, 487.

Sulzbacher, W. L., Gaddis, A. M. and Shamey, J. 1965. Die beziehung swischen spezifischen carbonylverbindungen und ranzigem geschmack. Abstract. Die Fleischwirtschaft 2, 143.

Sulzbacher, W. L., Gaddis, A. M. and Shamey, J. 1964. The relationship of specific carbonyl compounds to rancid flavor. 10th European meeting of Meat Research Workers. Roskilde, Denmark, August 10-14.

Zipser, Marelynn W., Kwon, Tai-Wan, and Watts, Betty M. 1964. Oxidative changes in cured and uncured frozen cooked pork. Journal of Agricultural and Food Chemistry 12, 105-109.

Gaddis, A. M., and Ellis, R. March 16, 1965. Use of Girard T reagent in quantitative aliphatic n-aldehyde determinations. U. D. Patent 3,173,761.

Meat Composition and Quality

Ackerman, S. A. 1965. Lightweight lantern slides. J. Chem. Ed. 42, 151.

Penny, I. F., Voyle, C. A. and Laurie, R. A. 1964. Some properties of freeze-dried pork muscles of high or low ultimate pH. Journal of the Science of Food and Agriculture 8. 559-565.

McClain, P. E., Mullins, A. M., Hansard, S. L., Fox, J. D., and Boulware, R. F., 1965. Acid and salt soluble collagen in bovine muscle. Proc. Soc. Exptl. Biol. Med. 119, 492.

Swift, C. E., and Sulzbacher, W. L. 1963. Theory of emulsions. Physical and chemical makeup of sausage emulsions. Proc. 16th Reciprocal Meat Conf. Stillwater, Okla., 69-81.

Swift, C. E. 1965. Processing research in USDA meat investigations. Western Meat Ind. 11, 16.

Walters, C. L., and Taylor, A. McM. 1964. Nitrite metabolism by muscle *in vitro*. Biochim. et Biophys. Acta 86, 448-458.

AREA NO. 4 - ANIMAL FATS AND OILS--INDUSTRIAL UTILIZATION

Problem. The 4-1/2 billion-pound-per-year output of inedible fats is one of the major products of the livestock industry. It also is one of major concern, because while production of animal fats has more than doubled in the last 15 years, its principal outlet (in soap) has declined sharply, and is still declining.

The best answer to the question of what to do with huge amounts of fats is to find new uses through utilization research. Already utilization research has played a leading role in finding new uses for over 1 billion pounds of animal fats, and thus helped retain markets for fats. Use of fat in animal feed which was developed through research, has now become the number one domestic use of inedible fats. There is need, however, for new uses not merely to retain or defend markets, but to expand them, and to upgrade the value of animal fats. The organic chemical industry presents a good opportunity for expanded markets, producing as it does a multitude of products--polymers, plasticizers, insecticides, herbicides, lubricants, paper chemicals--totaling 10 billion pounds. Animal fats possess "built-in" properties which make them potentially useful as raw materials to the chemical industry, but research must be done to realize this potential.

An increase of 1 cent per pound in the value of inedible animal fats would provide an additional revenue of $40 million of the livestock industry. This additional revenue will help the industry and growers in the same way as revenue from other animal products and by-products.

The attainment of an increase in the monetary returns from livestock requires both applied and basic research. Applied research is the forerunner of commercial practice and is an indispensable element in successful development. But applied research is based on the foundation of fundamental knowledge that is acquired through basic research, and represents the exploitation of this fundamental knowledge. The supply of fundamental facts about animal fats; composition, methods of separation of constituents, preparation of chemical derivatives of constituents and determination of their physical and chemical properties; must be maintained and expanded if applied research is to be most effective and fruitful. The need for basic research has been pointed out by the Commission on Increased Industrial Use of Agricultural Products, the National Agricultural Research Advisory Committee and by other responsible groups.

USDA AND COOPERATIVE PROGRAM

The Department has a broad program of basic and applied research at Wyndmoor, Pennsylvania, and at additional locations where contract and grant research is being carried out involving chemistry and physics, aimed at developing new and improved products from fats for use in industry. Total professional man-years (p.m.-y.) are 53.8. Of this, 11.9 p.m.-y. are devoted to studies on

chemical composition and the physical and chemical properties of animal fats at Wyndmoor. Studies involve fatty acid composition of animal fats using the latest advances in chromatography and other techniques; intra- and intermolecular structure of pure compounds and derivatives and factors that influence development of off-flavors in fatty foods. Research is underway at Villanova University, Villanova, Pa. with two contracts on special interrelationships within triglyceride molecules and on x-ray investigation of triglycerides and involves 0.7 p.m.-y. each. A research grant involving 1.0 p.m.-y. at Storrs, Conn. provides for the synthesis of pure glycerides. Additional research sponsored by the Department under P.L. 480 grants is now in progress at the University of Madrid, Spain, on the preparation of cocoa butter substitutes from animal fats, and at the Technical University, Gdansk, Poland, on a study of the kinetics and thermodynamics of fat autoxidation.

Research on improved polymers, plastics, resins and lubricants involving 16.3 p.m.-y. at Wyndmoor is conducted on the synthesis of organic compounds and the preparation of new products for use as plastics, plasticizers and lubricants. A research contract with the University of Arizona at Tucson involving 0.2 p.m.-y. deals with use of products derived from animal fats to synthesize plastics and other polymeric materials. In the current program an aspect of animal fat research is a contract with U. S. Industrial Chemical Company, New York on "Ethylene copolymerization with unsaturated fatty acids and gum naval stores." EU shares the effort to the extent of 0.4 p.m.-y. in cooperation with SU. Additional research underway at the University of Aix-Marseille at Marseille, France with P.L. 480 funds concerns preparation of hydroxylated derivatives of animal fats for use in industrial products such as plastics and lubricants.

At Wyndmoor 9.4 p.m.-y. are being devoted to research on development of improved synthetic detergents based on animal fats, which includes preparation, testing of detergent power, and measurement of biodegradability of α-sulfo fatty acids and their esters, tallow alcohol sulfates and other fat derived materials. A research contract with Lehigh University, Bethlehem, Pa. involving 0.5 p.m.-y. deals with interfacial absorption characteristics of fatty acids. The high pressure hydrolysis of animal fats to alcohols without simultaneous chain saturation will be investigated under a research contract with Swift and Company, Chicago, Ill. Additional research sponsored by the Department under a P.L. 480 grant is now in progress at the University of Bombay, Bombay, India on the preparation and properties of long chain sulfated monoglycerides.

Exploratory investigations of new chemical derivatives of animal fats for use as chemical intermediates for industry involves 10 p.m.-y. at Wyndmoor. A research contract on the chemical and physical characteristics of organic peroxides involving 0.7 p.m.-y. is in progress at the University of Pittsburgh, Pittsburgh, Pa. A research grant with the Hormel Institute of the University of Minnesota at Austin, Minnesota involving 0.6 p.m.-y. provides for the investigation of the ozonization of animal fats.

PROGRAM OF STATE EXPERIMENT STATIONS

A limited program of work directed to the utilization of fats and oils is in progress at the State Stations. More complete knowledge of the molecular structure and composition of the component glycerides is being developed and related to flavor stability. Chemical transformations are under investigation to minimize polymerization and conjugation during isomerization processes leading to industrially modified fats and oils. Studies continue on the nutritional quality of fats to assess the vitamin E content of diets low in cholesterol and poly-unsaturates. One research group is exploring the role of lipids in maintaining the beat of heart cells in vitro. In another study the metabolism of trans fatty acids, which occur in most edible hydrogenated fats, is being evaluated. Still another study deals with the chemical stability of frying fats, including lard, vegetable shortenings, and mixture of animal fats with hydrogenated vegetable oils to withstand repeated heating, re-use, and storage.

The role of anitoxidants and pro-oxidants is being investigated. Other research is directed toward identification of the components responsible for the flavors of auto-oxidized fats and the factors affecting their production. A precise quantitative method of analysis involving gas chromatography has been developed for C_6 and C_{22} fatty acids. Evidence for auto-oxidized flavor components of fats points to such aldehydes and ketones as cis, cis-3, 6-nonadienal and vinyl ethyl ketone. Synthesis of glycerides with controlled degrees of unsaturation have been worked out for testing changes in blandness.

Basic studies on minor constituents of animal and vegetable fats such as sulfo-lipids, phospho-lipids, and steroids are underway. Applied work to convert low grade fatty materials into industrially valuable products has led to a potentially commercial method for producing the more stable elaidic acid from oleic acid. Related studies on the trans-isomerization of ethyl linoleate are being pursued.

A total of 2.5 professional man-years is devoted to research on industrial utilization of animal fats and oils at the State experiment stations.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition, Physical Properties and Autoxidation.

1. Purification of lipids and determination of their structure. Through the use of polarized infrared spectroscopy, additional information was obtained on the structure of thin crystalline films of methyl esters of fatty acids and alkyl sulfonic acids; the data permit assignment of absorption bands in these compounds. Nuclear magnetic resonance spectroscopy was applied successfully to investigate the intramolecular hydrogen bonding of a model compound in polar solvents. Dielectric measurements were used to determine some thermodynamic properties of esters of fatty acids. X-ray diffraction methods have been applied to the determination of free urea in urea adducts of ethyl stearate.

The specificity of pancreatic lipase for the hydrolysis of the 1,3 ester groups was previously shown to be absolute and the technique to be useable as a criterion of purity for di- and triglycerides. A semi-micro hydrolysis technique has been developed in which the hydrolysis products are isolated by thin-layer chromatography and the fatty acid mixtures analyzed by GLC. By means of the semi-micro lipase hydrolysis technique, the glyceride distribution of depot fats from a series of animals was determined and a comparison was made of the glyceride distribution of normal lard and a randomly interesterified lard. Whereas lard has typically over 80% of palmitic acid in the 2 position, the randomly interesterified lard showed large decreases in the content of 2-palmitoyl triglycerides and large increases in the amount of symmetrical and unsymmetrical diunsaturated glycerides.

Under a P.L. 480 grant at the "Juan de la Cierva" Foundation for Applied Research, Madrid, Sapin, some progress has been reported in the development of suitable methodology in glyceride analysis for use in research on preparation from animal fats of substitutes for cocoa butter.

An unsymmetrical di-acid triglyceride has been successfully synthesized and determinations by x-ray intensity data and by unit cell dimensions have begun. A computer program has been prepared to analyze the x-ray data. Data of this kind will permit the exact determination of the structure of a single crystal.

Computers have been employed to calculate the effect of small changes in bond angles on intramolecular hydrogen bonding. These computations have demonstrated that the geometry of a large number of isomers of a molecule can be determined provided a high speed computer is available. A computer program has been developed for application to the calculation of spatial interrelations of triglyceride molecules.

2. Autoxidation. The autoxidation of methyl linoleate in emulsion has been studied in the presence of both cupric ion and its chelates with arginine and histidine. The results indicate that careful control of pH in an aqueous-fat system may be used to inhibit or accelerate the autoxidation of the fat. In the system so far investigated the pH range of 6-7 appears to be optimum for surpressing fat autoxidation. In the presence of a model system simulating oxidase activity the rate of oxygen uptake at pH 5.7 of a 0.1 M methyl linoleate emulsion was increased approximately 25 times. The model system contained ascorbic acid, ethylenediaminetetraacetic acid and ferric ions. As the ascorbic acid was destroyed the rate of oxygen uptake decreased from 25 to 4 times that of the control, indicating that ascorbic acid, often used as an anti-oxidant, may function as a pro-oxidant.

B. Improved Polymers, Plastics, Resins and Lubricants.

1. Polymers, plastics and resins. Internal plasticization was evaluated on copolymers of vinyl esters of cyclic and polychloro fatty acids with vinyl chloride. The lowest brittle point and most effective plasticizing was obtained with vinyl dichlorobehenate as the comonomer, followed in

effectiveness by vinyl stearate and vinyl dichlorostearate. Confirmation of internal plasticization of vinyl chloride by these vinyl comonomers enhances their prospects for commercial use.

Rigid urethane foams were readily prepared from polyols made by the reaction of 9,10-dihydroxystearic acid, either the erythro- or threo-isomer, with propylene oxide. Whereas all of the ethylene oxide adducts of erythro-dihydroxystearic acid, as well as the di- and octa-oxyethylates of the threo-isomer, were inconviently solid or semi-solid at room temperature, the propylene oxide adducts (1, 2, 4, 6 and 8 oxypropylene units) to either erythro-dihydroxystearic acid or the threo-isomer are liquids throughout both series. This feature, and the slower creaming times of propylene oxide adducts in reaction with isocyanate than ethylene oxide adducts, provides 9,10-dihydroxystearic acid derivatives which are convenient for use in 2-stage foam preparation and enhances the possibility of their commercial utilization. Measurement of the physical properties of the two rigid urethane series embodying oxypropylated derivatives of 9,10-dihydroxystearic acid has been completed except for the determination of compressive strength. The foams were found to contain over 80% closed cells, with densities ranging between 1.6 and 1.9 lbs./ft.^{3}.

Periodate cleavage of monoepoxidized methyl linoleate by two different versions of the periodate method indicate that there is no significant selectivity in the epoxidation reaction. The 9,10- and the 12,13-double bonds of methyl linoleate are epoxidized with equal ease, giving a product containing substantially equal amounts of the unsaturated epoxy derivatives, methyl coronate and methyl vernolate. Demonstration for the first time of methyl coronate as a synthetic product was made possible by the new variations of the periodate cleavage reaction.

In P.L. 480 supported research at Universite d'Aix-Marseille, Marseille, France, conversion of brominated fatty compounds to allylically hydroxylated derivatives was completed, the results were reported in two publications, and research was initiated on: allylic hydroxylation of unsaturated fatty acids with selenium dioxide; oxidation of allylically hydroxylated oleic acid to α,β-unsaturated ketones; and oxo addition of carbon monoxide and hydrogen to double bonds. For GLC identification of expected products, model branched long-chain derivatives were prepared from carboxystearic acid made by the ERRL method.

Evaluation was completed of the copolymers of N-n-butyl, octyl and octadecyl acrylamide with acrylonitrile with respect to torsional stiffness, temperatures and tensile properties. Tensile tests, which measure the effect of large chain deformations by relating stress to strain up to the break point, showed failure at low strain, characteristic of brittle materials. This affect was most pronounced with the octadecyl acrylamide. At temperatures greater than 100°C. where presumably the crystalline domains had melted, polymers were tough, resilient and flexible, the improvement in properties increasing with increase in amide chain length. Data obtained from the

evaluation of these copolymers indicate that long chain unsaturated acrylamides, such oleyl acrylamide, or long chain tertiary amides such as N-N-di-N-octadecyl acrylamide, will have a higher internal plasticizing efficiency and impart useful properties to acrylonitrile copolymers.

2. Lubricants. The dialkylphosphono adducts of unsaturated fatty acid esters or triglycerides from animal fat sources have been prepared and evaluated for use as lubricants or lubricant additives. From an economical viewpoint the naturally occuring triglycerides are the most attractive olefinic starting materials. However, when the phosphorus-containing triglycerides are prepared by the free radical addition of dialkylphosphonate to their olefinic centers by techniques entirely satisfactory for addition to monounsaturated esters, the time necessary for a complete reaction was increased by a factor of 5 to 6, to overcome this disadvantage a study was made of the time-temperature relationships for the reaction. It was found that an increase in the reaction temperature from 105°C. to 125°C. reduced the time for complete reaction 10-fold, or from 30-40 hours to 3-4 hours. When compounds prepared in this improved fashion were submitted to lubricant evaluation by anti-wear and extreme-pressure tests, they showed no deterioration in physical properties.

The preparation and lubricant evaluation of the products of the addition of dialkylphosphonates to unsaturated fatty esters and triglycerides was extended to the diesters of ethylene glycol. The products obtained are low melting solids with lubricant properties comparable to those found for the derivatives of triglycerides and monoesters.

Lubrication tests indicate that phosphorus-containing compounds derived from fats and oils resist wear, extreme pressure, and oxidation when used as base oils or as additives to both petroleum lubricants and synthetic diester lubricants.

C. Improved Synthetic Detergents.

1. Soap-detergent combinations from inedible animal fats. The esters of α-sulfo fatty acids have markedly different properties; the pelargonates are primarily excellent wetting agnets, the myristates, palmitates and stearates are lime soap dispersing agents; and these differences can result in specialized uses for individual esters. Eleven of the esters were prepared in a high state of purity and delivered to a contractor for measurement of physical-chemical characteristics. Preliminary information of the effect of pH and added electrolyte on surface tension values, and preliminary heat of wetting data have been obtained. The relation of interfacial absorption characteristics with structure of a compound and its performance as a detergent or wetting agent may be a guide to the further development of surface active agents and detergents from animal fats.

A series of monovalent and divalent metal salts of the alkyl esters of α-sulfo fatty acids were prepared for a study of structure-property relations.

Although all of the monovalent salts are sufficiently soluble in water, the potassium salts are the least soluble. Calcium and magnesium salts from gelatinous aqueous dispersions at higher concentrations, but they are readily soluble in organic solvents. Of 14 sodium salts evaluated for detergency, foaming, wetting, or lime soap dispersing properties, sodium methyl α-sulfopalmitate gave the best all around performance. All the esters are biodegradable.

Other compounds prepared are the ether alcohol sulfates and sodium alkenesulfonates. The latter were prepared by sulfonation of tallow-derived α-olefins. Sodium alkenesulfonates resemble sodium oleyl sulfate; they are readily soluble in water and have good detergent and surface active properties.

Ether alcohol sulfates of the formula $ROCH_2CH(OSO_3Na)CH_3$ were prepared by the oxypropylation and sulfation of saturated long carbon chain primary alcohols. The ether alcohol sulfates are excellent detergents and lime soap dispersing agents, being more readily soluble and more generally useful than saturated tallow alcohol sulfates. Both the ether alcohol sulfates and the sodium alkenesulfonates are easily biodegradable.

2. Biodegradability. The ease of biodegradation of tallow alcohol sulfates and esters of α-sulfo fatty acids were compared with linear (LAS) and branched-chain (ABS) alkylbenzenesulfonates under aerobic conditions representing the operation of an activated sludge sewage disposal system and under conditions representing anaerobic digestion in municipal sewage systems, septic tanks and cesspools. Tallow alcohol sulfates in particular, and certain esters of the α-sulfo fatty acids, are more readily tolerated than either linear or branched chain alkyl benzene sulfonates under both aerobic and anaerobic conditions of biodegradation.

A new method of evaluation of biodegradability based on chemical oxidation demand (COD) is under investigation. Whereas the currently used methylene blue test indicates degradation of the detergent to the point of loss of surface active properties, the COD measurement indicates extent of metabolism by microorganisms. From preliminary tests it appears that surface active properties are lost long before COD values reach a minimum.

D. New Chemical Derivatives.

1. Reactions and Reaction Mechanisms. Direct free radical addition of addends to unsaturated acids may lead to a variety of versatile and useful chemical derivatives. Investigations of the thermal free radical addition of acetic acid, acetic anhydride and ethyl cyanoacetate to methyl undecylenate and methyl oleate has been completed. A similar study on aromatic aldehydes has been initiated, but preliminary results indicate this reaction to be a complex one.

Improvements in the synthesis of diisopropenyl esters of dicarboxylic acids has improved the yield to about 60%, double that obtained initially.

Deuterium-labeling studies are being employed to investigate the mechanism by which gamma-stearolactone is formed in perchloric acid isomerization of oleic acid. The products are seperated by column chromatography and deuterium analyses carried out on the individual products. Mass spectromity is being used to determine the position of deuterium in the products. Results obtained support the hypothesis that the formation of gamma-stearolactone is mainly due to a series of intermolecular reactions of carboxyl with olefin to form ester (a dimer), a reaction which is reversible and results in double bond migration along the hydrocarbon chain. Ultimately the double bond reaches the vicinity of the carboxyl group on the same molecule, whereupon an intermolecular esterification results in the lactone formation.

The isomerization of oleic acid to gamma-stearolactone with sulfuric acid, the classical reagent, was reviewed thoroughly and systematically. None of the reactions produced gamma-stearolactone in yields comparable to those obtained from the perchloric acid isomerizations.

A program on the use of gamma-stearolactone as a chemical intermediate has been initiated. Alkali-fusion of gamma-stearolactone gave good yields of gamma-ketostearic acid.

The thiocyanation of methyl oleate was restudied with cis and trans-9-octadecene as model compounds for clarification of the mechanism. Nine distinct products of the reaction were obtained by column chromatography and countercurrent distribtuion.

2. Properties and structural characteristics of organic peroxides. Organic peroxides derived from animal fats are easily and inexpensively prepared, and they are labile compounds which undergo a wide variety of organic reactions. Basic information on the structure of these peroxide derivatives and thermodynamic data will aid in the development of reactions for converting peroxides of fats and their derivatives to commercially useful chemicals.

Results of the structure refinement of dibenzoyl peroxide obtained so far reveal an unusually interesting thermal behavior of the molecules in the solid state. The space group of the crystals is also compatible with a single enantiomorph suggesting that one single crystal will contain molecules of the same sense. The study of optical rotatory properties of benzoyl peroxide is contingent upon success in growing appropriate single crystals. This work is being conducted at the University of Pittsburgh under a research contract.

PUBLICATIONS AND PATENTS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition, Physical Properties and Autoxidation

Coleman, J. E., Hampson, J. W., and Saunders, D. H. 1964. Autoxidation of fatty materials in emulsion. II. Factors affecting the histidine-catalyzed oxidation of emulsified methyl linoleate. J. Am. Oil Chem. Soc. 41, 347-351.

Eddy, C. Roland. April, 1965. Flow diagrams for computation of spatial locations of atoms of a chain molecule undergoing intramolecular rotations. ARS-73-46, 16 pp.

Luddy, F. E., Barford, R. A., Herb, S. F., Magidman, P., and Riemenschneider, R. W. 1964. Pancreatic lipase hydrolysis of triglycerides by a semimicro technique. J. Am. Oil Chem. Soc. 41, 693-696.

Lutz, D. A., Hunter, J. J., and Eddy, C. R. 1965. Determination of free urea in the ethyl stearate adduct by x-ray diffraction. Anal. Chem. 37, 274-275.

Improved Polymers, Plastics, Resins and Lubricants

Ault, W. C., Micich, T. J., Stirton, A. J., and Bistline, R. G., Jr. 1965. Branched chain fatty acids and sulfonated derivatives. J. Am. Oil Chem. Soc. 42, 233-236.

Eisner, Abner, Perlstein, Theodore, and Ault, W. C. 1964. Methanesulfonic acid-catalyzed additions IV. Additions to methyl linoleate. J. Am. Oil Chem. Soc. 41, 557-558.

Jordan, E. F., Jr., and Wrigley, A. N. 1964. Homopolymerization of N-allyl-acetamide and N-allylstearamide. J. Polymer Sci. A, 2, 3909-3924.

Kenney, H. E., Komanowsky, Daria, and Wrigley, A. N. 1965. Reaction of dichlorocarbene with fats and oils. J. Am. Oil Chem. Soc. 42, 19-22.

Maerker, G. 1965. Determination of oxirane content of derivatives of fats. J. Am. Oil Chem. Soc. 42, 329-332.

Maerker, G., Haeberer, E. T., and Ault, W. C. 1964. Acid-catalyzed conversion of epoxyesters to hydroxyesters. J. Am. Oil Chem. Soc. 41, 585-588.

Marvel, C. S., Hill, J. C., Cowan, J. C., Friedrick, J. P., and O'Donnell, J. L. 1964. Preparation and polymerization of vinyl esters of cyclic and polychloro fatty acids. J. Polymer Sci. A, 2, 2523-2532.

Naudet, Maurice, Peretz, Arlette and Ucciani, Eugène. 1964. Sur quelques dérivés hydroxylés allyliques de la chaine octadécéneque. Bulletin de al Société Chimique de France 8, 1858-1864.

Naudet, Maurice, Ucciani, Eugene and Peretz, Arlette. 1964. Sur l'obtention de dérivés hydroxylés allyliques des chaînes grasses monoinsaturées. Revue Française des Crops Gras 5, 3-7.

Scholnick, F., Monroe, H. A., Wrigley, A. N., and Ault, W. C. 1965. Resins from Hydroxyphenylstearic acid. J. Am. Oil. Chem. Soc. 42, 23-24.

Walens, H. A., Koob, R. P., Ault, W. C. and Maerker, G. 1965. Rearrangement of epoxy fatty esters to keto fatty esters. J. Am. Oil Chem. Soc. 42, 126-129.

Ault, Waldo C., and Eisner, Abner. June 29, 1965. Process for addition of arythiols, phenols and phenyl ethers of unsaturated fatty acids. U. S. Patent 3,192,239.

Knight, Hogan B., and Swern, Daniel. June 15, 1965. Phosphonated oils and polyunsaturated esters. U. S. Patent 3,189,628.

Port, William S., and Monroe, Harry A. November 24, 1964. Process for preparation of vinyl esters. U. S. Patent 3,158,633.

Port, W. S., and Scholnick, F. September 1, 1964. Epoxy monomers and resins thereof. U. S. Patent 3,147,236.

Improved Synthetic Detergents

Bistline, R. G., Jr., and Stirton, A. J. 1964. Experiments in the formulation of heavy duty liquid detergents from tallow. J. Am. Oil Chem. Soc. 41, 654-656.

Cordon, T. C., Maurer, E. W., Weil, J. K., and Stirton, A. J. 1964. Biodegradation of esters of α-sulfo fatty acids in activated sludge. Developments in Industrial Microbiology 6, 3-15.

Maurer, E. W., Cordon, T. C., Weil, J. K., Nunez-Ponzoa, M. W., Ault, W. C., and Stirton, A. J. 1965. The effect of tallow-based detergents on anaerobic digestion. J. Am. Oil Chem. Soc. 42, 189-192.

Smith, F. D., Kenney, H. E., and Stirton, A. J. 1965. Isomeric arylstearic acids. J. Org. Chem. 30, 885-888.

Stirton, A. J., Smith, F. D., and Weil, J. K. 1965. Disodium 2-sulfohexadecyl and 2-sulfooctadecyl sulfates. J. Am. Oil Chem. Soc. 42, 114-115.

Weil, J. K., and Stirton, A. J. 1964. Biodegradation of some tallow-based surface active agents in river water. J. Am. Oil Chem. Soc. 41, 355-358.

New Chemical Derivatives

Belitskus, D., and Jeffrey, G. A. 1965. The crystal structure of peroxypelargonic acid. Acta Crystallographica 18, 458-463.

Beurskens, M. Sax. P., and Chu, S. 1965. The crystal structure of ortho-nitroperoxybenzoic acid. Acta Crystallographica 18, 252-258.

Roe, Edward T., and Swern, Daniel. February 9, 1965. Carboxylated derivatives of oleyl alcohol and method of preparation. U. S. Patent 3, 170,939.

Silbert, Leonard S., and Swern, Daniel. August 4, 1964. Preparation of aromatic peroxy acids. U. S. Patent 3,143,562.

AREA NO. 5 - HIDES, SKINS AND LEATHER - PROCESSING AND PRODUCTS

Problem. To maintain the utilization of animal hides and skins at a profitable level there is need to find new products and processes to provide outlets for about 11 million cattlehides that are now available in excess of domestic needs. The foreign markets that currently absorb these surplus hides are also threatened by the increased hide production and decreased per capita use of leather (the principal outlet for hides) that have dislocated U.S. markets and caused prices to drop so precipitously in the last 10 years. To meet this problem there is need for upgrading the quality of raw hides and skins, for reducing the costs of producing leather, and for developing new and nonconventional products from collagen. To achieve these objectives research is needed to develop improved curing processes and agents, more effective control measures for (ante mortem) defects such as grubs, brands and parasite damage, and improved methods of take-off. Fundamental research is needed on the composition of hides to provide basic information on the chemical, physical and physical-chemical properties and reactions of collagen and other hide components for use in studies on chemical modification and on the development of new and improved products and processes. Development of new, more rapid and economic processes for curing, handling, unhairing and tanning hides is needed to reduce the cost of producing leather. There is also need for research on the chemical modification of hide proteins to develop leather products with such improved "built-in" properties as increased resistance to wear, scuffing and deterioration from perspiration, enhanced washability, dry-cleanability and improved dyeability. There is also need for research on the physical and chemical properties of collagen to obtain information for use in dispersing and regenerating the fibrous structure without degrading its unique properties for developing nonconventional products that will provide new outlets and markets for hide proteins, with special reference to the field of edible products.

USDA AND COOPERATIVE PROGRAM

The Department is conducting a broad program of basic and applied research on hides, skins and leather at Wyndmoor, Pennsylvania, and at additional locations where contract and grant research is being carried out; this involves physicists, chemists, biochemists, microbiologists and leather technologists.

The Federal scientific effort devoted to the over-all program totals 29.8 professional man-years, as follows:

(a) Chemical and physical properties and structure of hides and collagen involve 8.6 p.m.-y. at Wyndmoor. These investigations encompass exploratory research on cattlehide components and basic research on the chemistry of collagen. One line of investigations is concerned with the isolation and identification of cattlehide components, their chemical and physical properties, and their organization within the hide structure as related to leather properties. The second part of the program deals with the forces that control

the stability and reactivity of collagen and the factors responsible for the unique physical properties of leather. Of special interest are the physical-chemical properties of collagen, its soluble components and its reaction products with modifying chemical agents. The University of Cincinnati, Cincinnati, Ohio, is conducting contract research on the noncollagenous proteins of cattlehides involving 0.4 p.m.-y.

Under a grant with Northwestern University, Evanston, Illinois, 0.9 p.m.-y. is being spent to study the physical properties of collagens.

In addition, research sponsored by the Department under the P.L.-480 program is in progress at: (1) University of Turku, Finland, on the fractionation of gelatin and soluble collagen; and (2) Central Leather Research Institute, Madras, India, on the reaction of polyphenolic tanning compounds with hide proteins (collagen);on the hydrothermal shrinkage of collagen and leather; and on the comfort properties of shoe leathers.

(b) Chemical modification of hides involves 9.0 p.m.-y. at Wyndmoor. This program is concerned with research on the reactivity of hide proteins with various organic and inorganic chemicals in the development of new products intended for specific uses.

In addition research sponsored by the Department under the P.L.-480 program is in progress at the British Leather Manufacturers Association, Surrey, Great Britain, for the study of chemically reactive compounds to improve leather stability.

(c) New and improved processing involves 8.6 p.m.-y. at Wyndmoor. Research is aimed at developing better methods for unhairing animal hides and skin, for imparting deterioration resistance to leathers, and for processing hides into leathers possessing special properties. The University of Cincinnati, Cincinnati, Ohio, is conducting research on the abnormalities of leather characterized by a depleted mushy texture involving 1.4 p.m.-y., and the Mid-west Research Institute has initiated studies on the dispersion of collagen for the development of new products involving 0.7 p.m.-y.

Additional research under P.L.-480 grants is in progress at: (1) Central Leather Research Institute, Madras, India, on the interrelation of hide quality with the rate of tanning and efficiency of tanning and on the rapid tannage of sole leather; and (2) Leather Research Institute, T.N.O., Waalvijk, Holland, on kinetics of chrome tanning.

(d) Utilization of animal protein residues involves research in cooperation with the National Renderers Association, who support one Senior Fellow and one Junior Fellow at Wyndmoor, Pa., to conduct basic studies on the evaluation of meat and bone meal fractions of residues from fat rendering operations. The Department of Agriculture participates in this program to the extent of supplying supervisory and research leadership amounting to 0.2 p.m.-y.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

State stations reported no research in this area.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical and Physical Properties and Structure of Hides and Collagen.

1. Chemical and physical properties. Observations have been made on the thermal behavior of collagen and leather through the use of differential thermal analyses. Information obtained not only provides a better understanding of the basic energy changes involved when hide materials undergo a change but are of immediate importance to applied problems involving the manufacturing (processing) of leather.

The mechanical properties of leathers have been investigated by means of a nondestructive dynamic test procedure. Comparative measurements of physical characteristics were made over the entire area of intact sides of leather which revealed a pattern of the variability of the properties within a side.

Continued progress has been made under a P.L.-480 grant at the University of Turku on the specific properties of collagen and gelatin that are applicable to the development of expanded uses of animal hides and skins. Starch gel electrophoresis has proved to be a powerful tool in the separation of most of the components from denatured collagen. Application of linear temperature gradient in the gel sheets has demonstrated how denatured collagen subunits change to the collagen-fold in this order with decreasing temperature: β_{11}, β_{12}, α_1, α_2. Studies have revealed that when collagen is hydrolyzed with pepsin, that two distinct units are split from the bulk of the collagen. Their source needs to be determined. The P.L.-480 grantee at the Central Leather Research Institute, Madras, India, has reported further progress on the mode of reaction of polyphenolic compounds with collagen. Purified tannin derived from mangrove penetrates the hide faster and gives higher hydrothermal stability than crude extracts. This has prompted the fractionation and evaluation of other tannins to establish the universality of this property.

Under a P.L.-480 project the Central Leather Research Institute, Madras, India, is studying the effect of tannery processes, such as liming, bating, pickling, tanning, fat-liquoring, etc. on the shrinkage temperature (T_s) and the accompanying dimensional changes. Specimens from each stage showed appreciable loss in area when the shrinkage temperature was exceeded. The change was least for limed stock; all of the others showed about a 10% loss, while pickled stock showed the most pronounced change of 30 to 40%. This Institute has recently initiated work under another grant on the factors affecting the comfort of leather apparel and footwear.

2. Microscopy. Progress has been made in the microscopy of the structural features of hides and skins and has yielded significant information on the disorganizations in the architecture associated with such defects as

veininess and mushy texture. Microscopy with polarized light and with phase systems offers a new dimension of observation and has revealed details of structure not seen under ordinary procedures. Use of ultrathin wedges has shown that structural features can be observed both under visual and electron techniques. This overlap makes possible a continuous range of magnification from a few powers up to 100,000 times in controlled steps so that identity of features is not lost. With these improved techniques for determining hide architecture more progress toward identifying the chemical and physical causes of hide defects can be made and the economic losses caused by them eliminated.

3. Collagen dispersion. Viscosity measurements on preparations of calfskin collagen solubilized in inorganic buffers has demonstrated a"non-Neutonian" behavior. Correction for this effect may more than double the presently accepted value for the intrinsic viscosity of collagen. The fundamental information resulting from this study should enhance the understanding of the physical chemistry of solubilized collagen and presents a new point of view regarding the viscometry of systems composed of large macromolecules. Since the continued profitable utilization of animal hides will depend to some extent upon the development of new products from collagen, information on its behavior toward dispersing agents and to changes in influence of ions will aid in the development of a process for the dispersion and reconstitution of collagen and may lead to new items containing its characteristic properties.

Behavior of collagen and gelatin in nonaqueous systems are under investigations at the Northwestern University School of Medicine. Physical measurements on the interactions of various nonaqueous solvent pairs indicate the presence of strong hydrogen bonding. These solvent pairs are being utilized to study the behavior of synthetic polypeptides of known composition and subsequently will be used for solubilizing collagen.

4. Effect of hide components on properties. A study of the distribution of the lipid classes in each of five stratigraphic layers for eleven areas whose combined area totaled over two-thirds of the area of a steer hide has shown that the variability of the lipid content of a hide is more complex than expected. Each of the five lipid classes has its own pattern of variability both areawise and through the thickness of the hide. This is one indication of the extreme complexity of the raw material used by tanners.

In contract work at the University of Cincinnati it has been shown that the noncollagenous proteins are extracted to varying degrees from hides by brines of different concentrations. This may explain the many conflicting reports in the trade about the leathermaking qualities of commercial brine-cured hides. A 1% brine appears to be the most efficacious extractant from the standpoint of ease of use and quantity of proteins extracted. Hides extracted with different brines are being tanned into shoe upper leather and the data obtained on the changes produced by differences in brining operations will be useful in developing recommendations for improving the quality of brined hides.

B. Chemical Modification.

1. Improved water repellency for leather. Previous reports have outlined progress in this area. A large-scale test has now been made with 12 commercial sides. It has been found that retannage of chrome leather with glutaraldehyde and lubrication with an alkenyl succinic acid improves the efficiency of subsequent water repellent treatments with silicone, Scotchgard, or Quilon. The most important feature of this development is the novel process for applying the alkenyl succinic acid to leather from an emulsion. These studies have demonstrated that smaller amounts of water repellent can be used and still retain effective water repellency.

2. Mannich reaction. The amount and kind of reactive groups present in hide protein limit the scope of its chemical reactivity. However, the Mannich reaction, which involves interaction between amine, formaldehyde, and a compound with an active hydrogen, can broaden this scope of reactivity. Hide protein (source of amino groups) was treated with formaldehyde and a wide variety of compounds with active hydrogen. The most promising of the latter was malonic acid. Studies of model system revealed that treatment of glycine, β-alanine, and α-aminobutyric acid with formaldehyde and malonic acid gave complex unresolved mixtures. However δ-aminovaleric and ε-aminocaproic acid both gave insoluble and apparently pure compounds whose structures (through infrared and NMR spectra) are indicated to be $(HO_2C)_2C\underline{/}CH_2NH(CH_2)_nCO_2\underline{H/}_2$, where n = 4 and 5, respectively.

3. Aldehyde leathers. A spectrophotometric method for determination of glutaraldehyde in solution, based on the 2,4-dinitrophenylhydrazone derivative, was developed. This procedure is rapid and specific for carbonyl groups and permits analysis for glutaraldehyde in the presence of many substances used in tanning of leather.

A method for the direct determination of bound glutaraldehyde in leather has been evolved. This is based on the discovery that hydrolysis of glutaraldehyde-tanned hide protein produces a substance absorbing at 265 mμ in the ultraviolet region. From a study of glutaraldehyde balance it was shown that absorbance at 265 mμ correlated with uptake of glutaraldehyde.

Early investigations demonstrated glutaraldehyde-tanned leather to have superior resistance to deterioration by perspiration and hot, soapy water. Washability, a property not enjoyed by leather, would enhance the utilization of hides and skins. A study on glutaraldehyde garment leathers showed the improved stability of this tannage to washing and offers considerable promise in stabilizing chrome leather. However other problems, such as wash-fast dyes, oils, and finishes, are confronted and need investigation. Research has begun on evaluating reactive dyes, such as the Procion dyes, in this application.

Studies have demonstrated the tanning ability of Dextraset UN under mildly alkaline conditions. This material, a cyclic derivative based on urea, formaldehyde, and methanol, produces a white leather. Further work is

necessary to define the optimum reaction conditions and to evaluate fully the properties of the leather. Other methylol derivatives have also been studied including those of imidazolidone, ethyl carbamate, urea, ethylene urea, and hydroxyethyl carbamate. In general the treatments with these methylol derivatives were similar to those with formaldehyde but with lower reactivity and shrinkage temperature.

The stability of hide protein can be increased by introduction of cross links through modification with polyfunctional reagents. Research under a P.L.-480 grant with the British Leather Manufacturers Research Association has been initiated to study mechanisms by which leather can be stabilized against deterioration from sweat and other chemicals. Exploratory studies with glutaraldehyde, formaldehyde, glyoxal, cyanuric chloride, acid chlorides, and a vinyl derivative indicates that glutaraldehyde is the most active cross-linking agent. Glutaraldehyde labeled with isotopic carbon was synthesized and used in studies on the mechanism of its reaction.

C. New and Improved Processes.

1. Enzymic unhairing of hides and skins. It has not been possible to produce commercially satisfactory shoe upper leather from enzyme unhaired hides in the complete absence of the conventional alkaline swelling of the collagen. Lime has been used after enzyme unhairing to produce reasonably good leather in several instances, although the results have not been wholly consistent on all lines of leather in different tanneries. While the use of lime increased the sewage disposal problem it has been possible to eliminate the most objectionable constituent of an unhairing liquor, namely, the inorganic sulfide. Commercially acceptable sole and handbag leathers have been produced from enzyme-unhaired hides without the use of any lime. An industrial firm is now conducting applied research in this area as a result of our contributions. The project will be terminated this year.

2. New tanning processes. The use of glutaraldehyde is expanding rapidly to improve the perspiration resistance and alkali stability of leather. Special applications such as the tanning of woolskin hospital bed pads, cattle belly work glove leather and deerskin garment leather, the latter in cooperation with RAD, are under investigations. Tanners are now so well aware of the practical advantages of the glutaraldehyde tannage that they are finding applications and are even adding lines of leather new to them.

3. Abnormalities of leather characterized by a depleted, mushy texture. Continuing progress under contract with the Tanners' Council Laboratory of the University of Cincinnati has established that a hide defect known in the trade as "pulpy butts" is largely, although not exclusively, confined to heavy, plump Hereford hides. Spready Hereford and plump Angus hides are almost free of this defect. Histological sections revealed that there is a much more vertical "angle of weave" in the fiber structure of the weak areas. Analyses of strength data indicate that the stitch tear test may be as reliable as the tensile test in predicting incidence of defective leather. Also stitch tear could be performed with greater facility in a slaughterhouse.

4. A study of the kinetics of chrome tanning. This research is being carried out under a P.L.-480 grant at the Lederinstituut T.N.O., Waalwijk, Holland. Initial work on pure basic chromium sulfate solution shows that the amount of chromium retained by the hide increases continuously, but the effect of time decreases with decreasing chromium concentration. Basicity of the liquor is important only during the early stage of tanning. The pyridine method for determining ionic sulfate is unreliable for different basicities and time of ageing of the tanned leather. Acetone dehydration of the tanned hide powder arrested the changes and will be used at different time intervals to determine the different states of the chromium sulfate.

5. Relationship of hide quality to tanning rate. The Indian grantee of this P.L.-480 project has shown some interesting differences between hides taken from fallen animals and those that were slaughtered. The fallen hides gave a positive test for delayed cure by the gelatin film procedure. The fallen hides showed excessive veininess, blood occlusion and grain damage. No differences could be detected internally by microscopy. The chrome tanning rate was not greatly affected by the hide quality. However, finished leather from the fallen hides was downgraded.

6. Investigations on "red heat." The Stazione Sperimentale per I'Industria delle Pelli e delle Materie Concianti of Naples, Italy, has concluded studies under a P.L.-480 grant in the causes and prevention of "red heat" damage that occurs on salted American packer hides received abroad. Pilot scale tests by Armour and Company revealed that the four germicides evaluated did not completely prevent the development of red heat when added to the curing salt. Subsequent studies by the grantee revealed that the loss of germicidal potency was caused by the U.S. commercial practice of employing a mixture of used and new salt for curing. Laboratory tests proved that the excessive inoculum carried by the used salt exceeded the germicidal power of the chemicals employed.

D. Utilization of Animal Protein Residues.

Investigations supported by the National Renderers Association are concerned with evaluating the quality of meat and bone meals from fat rendering residues by characterization of the proteins present. Amino acid analysis of the numerous fractions prepared from representative meals has shown that: (1) all the globular protein fractions are similar; and (2) the gelatin or nondialyzable fraction from autoclaving contains many amino acids not derived from collagen. The presence of amino acids not originating from collagen or gelatin indicates that autoclaving to remove the gelatin also removes other protein fragments and cannot serve as a procedure for enriching the meals. Early in the year the Fellows resigned, and the National Renderers Association have asked that new personnel be recruited for continuation of the program.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical and Physical Properties and Structure of Hides and Collagen

Cordon, T. C. 1965. Control and estimation of the fungal resistance of leather. (A.C.S. Monograph) Chemistry and Technology of Leather 4, 333-368.

Fein, M. L., Harris, E. H., Jr., and Calhoun, R. R., Jr. 1965. Report on shrink temperatures - determination of Ts on suspended leather specimen. J. Amer. Leather Chem. Assoc. 60, 15-30.

Gruber, H. A., Viola, S. J., and Mellon, E. F. 1965. The composition of tight hair spots. J. Amer. Leather Chem. Assoc. 60, 96-108.

Heikkinen, E., Mikkonen, L., and Kulonen, E. 1964. Age factor in the maturation of collagen. Cross-links in heat-denatured collagen in tail tendon and skin of rat. Experimental Gerontology 1, 31-36.

Isomaki, H., and Kulonen, E. 1963. Effect of lathyrism on nucleic acids and subcellular particles of the experimental granulation tissue. Acta Pharmacol. et Toxicol. 20, 227-232.

Juva, K., and Kulonen, E. 1964. Studies in experimental lathyrism III. Effect of lathyrism on the imino acids of chick embryos. Biochemical Pharmacology 13, 85-88.

Kulonen, E. 1963. Kollageenin biokemiasta. Duodecim 79, 723-730.

Kulonen, E., Lampiaho, K., Ahonen, J., and Kari, A. 1964. Limiting factors in collagen synthesis in granuloma slices. Abstracts from Sixth International Congress of Biochemistry, New York City, N. J., July 26-August 1.

Majaniemi, T., and Kulonen, E. 1964. In vitro experiments with granulation tissue on the maturation of collagen. Acta Pathologica et Microbiologica Scandinavica 60, 187-193.

Nanto, V., Maatela, J., and Kulonen, E. 1963. Separation of the subunits of denatured collagen and gelatins with starch gel electrophoresis. Acta Chemica Scandinavica 17, 1604-1608.

Nikkari, T., and Kulonen, E. 1964. Studies in experimental lathyrism IV. On reconstitution of neutral salt-soluble collagen from lathyritic rats. Biochemical Pharmacology 13, 89-93.

Pikkarainen, J., and Kulonen, E. 1964. Effect of pH and other conditions on the fractionation of gelatins in carboxymethylcellulose columns. Suomen Kemistilehti B37, 31-33.

Santhanam, P. S., Ghosh, D., and Nayudamma, Y. 1964. Studies on the fixation of goran tannins on chemically modified collagen. Leather Science 11, 215-221.

Witnauer, L. P., Bloom, F. W., Killen, D. R., and Riser, G. R. 1964. Measurement of the density of hide and leather. J. Amer. Leather Chem. Assoc. 59, 682-690.

Witnauer, L. P., and Palm, W. E. 1964. The flexibility of leather. J. Amer. Leather Chem. Assoc. 59, 246-258.

Witnauer, L. P., and Wisnewski, A. 1964. Absolute measurement of shrinkage temperature of leather by differential thermal analysis. J. Amer. Leather Chem. Assoc. 59, 598-612.

Chemical Modification

Bowes, J. H., Cater, C. W., and Ellis, M. J. 1965. Determination of formaldehyde and glutaraldehyde bound to collagen by carbon-14 assay. J. Amer. Leather Chem. Assoc. 60, 275-285.

Bowes, J. H., and Cater, C. W. 1965. Action of perspiration on leather. III. A laboratory test for the assessment of the relative stability of leathers to perspiration. J. Soc. Leather Trades Chemists 49, 189-195.

Filachione, E. M., Fein, M. L., and Harris, E. J., Jr. 1964. Tanning with glutaraldehyde IV. Use in side leather. J. Amer. Leather Chem. Assoc. 59, 378-402.

New and Improved Processing

Cordon, T. C., Jones, H. W., and Naghski, J; and Giffee, J. W. (American Meat Institute Foundation). 1964. Benzalkonium chloride as a preservative for hides and skins. J. Amer. Leather Chem. Assoc. 59, 317-326.

Formisano, M. 1964. Un quadriennio di richerche sul "calore rosso" delle pelli bovine salate. Estratto dai Bollettini della Stazione Sperimentale per l'Industria delle Pelli e delle Materie Concianti 40, 2-3.

Happich, M. L., Palm, W. E., Windus, W., and Naghski, J. 1965. The compatibility of glutaraldehyde and wattle in drum tanning. J. Amer. Leather Chem. Assoc. 60, 223-233.

Happich, M. L., Willard, H. J., Naghski, J., and Windus, W. 1964. Canaigre tannin - high yielding strains, seasonal variation, and germ plasm bank. J. Amer. Leather Chem. Assoc. 59, 475-486.

Happich, W. F., Happich, M. L., Windus, W., Palm, W. E., and Naghski, J. 1964. The tanning of shearlings with glutaraldehyde. J. Amer. Leather Chem. Assoc. 56, 448-461.

New and Improved Processing

Windus, W., and Hannigan, M. V. June 15, 1965. Tanning with a hemiacetal and resorcinol or pyrogallol solution. U. S. Patent No. 3,189,403.

AREA 6. POTATOES - PROCESSING AND PRODUCTS

Problem. The potato industry, faced with a continuing decline in the consumption of fresh potatoes, has turned to and is becoming more and more dependent upon the development of new and improved processed products to maintain markets and avoid recurring economic disasters. Crop perishability, fluctuations in supply, and inelasticity of demand, result in wide price swings with even slight surpluses. Depressive lows are moderated by advance contracting by processors prior to harvest in producing areas having a substantial processing industry. However, in many processing areas, processing has not yet been developed and vulnerability still exists and is exaggerated by the growing competition of processed potato and other vegetable food products. If processing is to expand rapidly enough to offset progressive decline in fresh potato consumption, a continuing improvement in currently produced products and development of new products is clearly required.

Lack of adequate knowledge concerning the chemical constituents, physical properties, and enzyme systems in potatoes is limiting development of new and improved products and processing methods. Basic research on composition is needed to provide fundamental information on which an applied research program can be systematically and effectively built. Recently developed techniques make possible the isolation, characterization, and analysis of constituents responsible for flavor, color, odor, and texture of many processed food products which were not available to research in the past. Application of such techniques to potatoes and potato products should make possible the improvement of the quality of present products, both freshly processed and following storage, and provide a basis for technological and engineering studies in new product development.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program of basic and applied chemical and engineering research on studies related to processing. The work of the EURDD, involving the services of chemists, biochemists, food technologists and chemical engineers at Wyndmoor, Pennsylvania,and East Grand Forks, Minnesota, is conducted in cooperation with several Agricultural Experiment Stations which supply potatoes of known cultural history and with the marketing research facilities of the Department. The chemical research program includes: isolation and characterization of the amino acid-sugar intermediate compounds responsible for the browning of chips and French-fried potatoes during processing; studies on lipids, which are believed to play an important role in storage stability of processed potato products, particularly dehydrated products; isolation and characterization of the proteins, which are important from a nutritional aspect and from their possible involvement in textural and processing characteristics; elucidation of the causes of after-cooking discoloration and isolation and characterization of the pigment formed; methods of predicting textural characteristics of potatoes for French-fried potatoes. The Eastern Division's engineering and development research

program seeks to improve the quality, nutritive value and storage stability of dehydrated potato products and to develop more convenient types of dehydrated products, such as "instantized" pieces that rehydrate and cook quickly. The Red River Valley Potato Processing Laboratory, East Grand Forks, Minnesota, has been established to conduct investigations relating variety and other raw material characteristics to quality of established forms of processed potatoes. This Laboratory is operated jointly by the Red River Valley Potato Growers Association, University of Minnesota, North Dakota State University, and the Agricultural Research Service with the Engineer-in-charge reporting to Wyndmoor. The Federal (EU) scientific effort devoted to this area totals 12.4 professional man-years. Of this total, research on chemical composition as related to processing characteristics comprises 9.2 p.m.-y. Research on new and improved processing technology amounts to 3.2 p.m.-y., including 2.0 p.m.-y. for the Red River Valley Processing Laboratory.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

State stations have a continuing long-term program of basic and applied research related to potato utilization. These studies cover the spectrum of problems ranging from the effect on quality of production practices such as variety, fertilization and management, to shelf-life, quality and flavor of processed products.

Mechanical harvesting procedures and storage conditions affect potato composition and in turn suitability for processing. Relating variety, raw material characteristics and storage conditions to quality of the various forms of processed potatoes receives attention at a number of stations. Much effort continues to be given to careful composition studies, particularly those relating to nitrogenous constituents, lipids, amino acids and sugars.

The potato industry is becoming more and more dependent upon development of new and improved products to maintain per capita consumption levels. Product research is directed to solution of problems of texture, sloughing, discoloration and processing procedures such as deep fat frying.

Newer techniques of flavor research are being applied in the search for an understanding of the flavor and flavor changes in potato products. For example, the volatile flavor compounds associated with fresh and stale potato chips are being investigated in order to better understand the changes which occur in these compounds during storage.

The utilization program also includes studies designed to find new and improved used for sweet potatoes. Studies related to determining suitability for canning, freezing and dehydration are continuing. New research is related to developing improved methods for drum drying sweet potatoes and for drying purees of high solids content. Problems of packaging, additives, storage stability and preparation and use also are under investigation.

The number of professional man-years devoted to potato utilization research is 9.4.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition as Related to Processing.

1. Nitrogenous constituents. Analyses for individual extractable amino acids for the 1961, 1962 and 1963 crop samples of Katahdin, Russet Burbank, Kennebec, Red Pontiac and Cobbler varieties grown in Idaho, Maine, New York, Pennsylvania, Red River Valley, and Wisconsin, have been completed. Statistical analysis for each year and for the three years are now in process in preparation for publication.

Analysis of the 1960 Katahdin crop from Maine and New York has verified the extractable and total nitrogen data obtained with the 1959 crop. Both total nitrogen and extractable nitrogen vary inversely with the total solids when calculated on a dry weight basis. However, on a fresh basis, the amount of nitrogen laid down per gram of tissue is reasonably constant for each individual year. Comparison of the two years, indicates considerable difference in the nitrogen level even though the environmental conditions were remarkably the same. This phase of the experimental work has been completed.

2. Pigments formed during frying of chips. Preliminary studies indicate losses of reducing sugars up to 67% and of amino acids up to 52% when extreme frying conditions are employed. The amino acid-sugar intermediates can be determined by means of the automatic amino acid analyzer. This indicates that ion-exchange methods can be employed for isolating these compounds.

3. Basic studies on potato lipids. Methods have been developed for following the total fatty acid content of potatoes in samples from the raw, immature potato, through processing into flakelets and storage. These include hydrolytic and gas chromatographic methods.

4. Basic studies on the proteins of potatoes. Through gel electrophoretic methods, a minimum of fifteen different proteins are indicated. Comparison of extracts of several varieties indicates little qualitative differences in the proteins but a rather definite difference in the quantitative values. This will aid in selection of varieties for ultimate isolation and characterization of individual proteins.

5. Basic studies on the after-cooking discoloration pigment. Studies on the extraction of the pigment from discolored potatoes have shown an apparent fractionation when progressively increasing concentrations of ethanol are used. It is not yet known whether this is an actual fractionation of different compounds or an apparent fractionation due to solubility. Chromatographic studies are presently in progress.

B. New and Improved Processing Technology.

1. Texture and color of French fries. An objective test, using the Lee-Kramer Automatic Recording Shear Press, is being investigated as a means of predicing quality of the processed product. Work to date has been on

interpretation of the curves produced and relating the pressure changes recorded with the actual events occurring during the shear operation. This work is being done in cooperation with the Horticultural Branch of ARS.

2. New puffing gun design. A gun for the explosion puffing of partially dehydrated fruits and vegetables has been designed. It is also admirably suited to potatoes. Construction drawings have been provided to industry and one company has already received several orders for commercial units. The new gun employs superheated steam in addition to external heat. This results in less heat damage to the product and much higher production rates.

3. New Red River Valley Laboratory in operation. Work is now underway at the Red River Valley Potato Processing Laboratory at East Grand Forks, Minnesota, to determine the effect of variety and cultural practices on the quality of processed potato products. Kennebecs and Pontiacs harvested at two dates, stored and reconditioned have been analyzed for reducing sugars, invertase, pH, phosphorylases, phosphatase and solids content. Pilot plant evaluation of the raw material has thus far been done by conversion to potato chips. The work has not yet progressed sufficiently far to justify conclusions.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition as Related to Processing

Fitzpatrick, Thomas J., Talley, Eugene A., and Porter, William L. 1965. Preliminary studies on the fate of sugars and amino acids in chips made from fresh and stored potatoes. J. Agr. & Food Chem. 13, 10-12.

Heisler, E. G., Siciliano, J., Woodward, C. F., and Porter, W. L. 1964. After cooking discoloration of potatoes. Role of the organic acids. J. Food Sci. 29, 555-564.

Porter, W. L., Fitzpatrick, T. J., and Talley, E. A. 1964. Studies of the relationship of specific gravity to total solids of potatoes. Am. Potato J. 41, 329-336.

Porter, W. L. and Talley, E. A. 1964. Digitizer for application of computers to automatic amino acid analysis. Anal. Chem. 36, 1692-1693.

Talley, Eugene A., Fitzpatrick, Thomas J., and Porter, William L. 1964. Chemical composition of potatoes. IV. Relationship of the free amino acid concentrations to specific gravity and storage time. Am. Potato J. 41, 357-366.

New and Improved Processing Technology

Heiland, Wolfgang K., and Eskew, Roderick K. 1965. A new gun for explosive puffing of fruits and vegetables. U. S. Agricultural Research Service, ARS-73-47, 7 pp.

AREA NO. 7 - VEGETABLES - PROCESSING AND PRODUCTS

Problem. Vegetable growing occupies over 3 million acres, with a yearly farm value of a billion dollars. Utilization as processed rather than fresh vegetables provides a constant source of supply with less price fluctuation. Basic compositional research is needed to provide knowledge to constituents responsible for color, flavor and texture of vegetables and the changes these constituents undergo during processing, storage, and distribution. There is also need for application of these results to developmental research on new products and new and improved processing technology. Consumer preference is shifting to "convenience" foods. An even greater emphasis on quickly prepared foods is evident in modern military feeding where high bulk density, nonrefrigerated, and rapidly rehydrating products are of primary importance.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program employing chemists and chemical engineers in basic and applied research on vegetable processing and products. The Federal work is conducted at Wyndmoor, Pennsylvania. The scientific effort assigned to this area totals 5.9 professional man-years and is currently engaged in research on new and improved products and processing technology.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

State stations conduct a broad program of basic and applied research on vegetable processing and products in order to maintain the place of vegetables in the diet and to overcome problems associated with the perishability and seasonality of vegetable crops. Research on the adaptability and evaluation of vegetable varieties for processing is a continuing service to vegetable breeding programs. Each promising introduction or variety is evaluated with respect to processing yield and characteristics.

Increased public concern with protection of the food supply from pesticide residues has resulted in initiation of three regional projects to study reduction or removal of residues from food products. Since commercial food processing and preparation procedures vary, the effect of these processes on residue removal is being evaluated. There is also an urgent need to develop rapid, sensitive methods for routine determination of pesticide residues on foods undergoing commercial processing. Data relative to chemical form, distribution and persistence is being amassed. Vegetables are included in the crops being studied. One objective of regional project NEM-30 involves study of the basic physiology and chemistry of changes taking place in post-harvest handling and processing.

Characterization of raw materials extends to consideration of the effects of various production variables upon processed product quality. Mechanical harvesting and the associated effects upon ultimate processed product quality are receiving increased study. The degree of correlation or association

between color, flavor and texture in fresh and in processed items continues to be a major concern.

Basic chemical and physical properties of vegetables are related to product acceptance and quality. Research on vegetables in this area ranges from standard composition studies to highly specialized analysis for mineral components. Research aimed at describing the biological changes that occur in vegetables at different stages of maturity continues. The role of enzymes and pigments in vegetables also receives continuing study.

Basic microbiological research centers around the high resistance of bacterial spores to heat and the adverse effects extreme thermal process requirements have on canned vegetables. Microbiological studies extend from determination of thermal process requirements to study of the natural flora of fresh vegetables. The radioresistance of bacterial spores and use of combined antibiotics and heat are carefully researched.

Processing technology research is directed to studies of freeze-drying, brining, canning, fermentation, hydro-cooling and controlled atmosphere methods. The comprehensive study of the effects of controlled or modified atmosphere on the biochemical, physical and quality characteristics of various vegetables continues.

New or improved product development research seeks to improve or perfect such items as "quick cooking" peas and beans, beet chips, various snack items, soups; and new sauerkraut products. Basic information relative to composition, nutritive value and functional properties is emphasized.

The total station research effort devoted to vegetable processing and products is 64.1 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. New and improved products.

1. Quick-cooking dehydrated vegetable pieces of high density. Because of their expanded, porous, structure, explosion puffed dehydrated vegetables tend to be more bulky than their conventionally dried counterparts. It has recently been found that this bulkiness can be eliminated by compressing the pieces between rolls immediately after puffing while they are still moist. On final drying the compressed pieces have a bulk density no greater than conventionally hot air dried pieces of the same original size and they rehydrated just as quickly and regain their shape as though they had not been compressed. This finding will significantly reduce the cost of the finished packaged product.

B. New and improved processing technology

1. New puffing gun design. A gun for the explosion puffing of partially

dehydrated fruits and vegetables has been designed. Construction drawings have been provided to industry and one company has already received several orders for commercial units. The new gun employs superheated steam in addition to external heat. This results in less heat damage to the product and much higher production rates.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

New and Improved Products

Greenspun, Reba B., Kelley, Edward G., and Porter, W. L. 1964. Chips and other food items from deep-fat fried mushrooms. U. S. Agricultural Research Service, ARS 73-44, 8 pp.

New and Improved Processing Technology

Heiland, Wolfgang K., and Eskew, Roderick K. 1965. A new gun for explosive puffing of fruits and vegetables. U. S. Agricultural Research Service, ARS 73-47, 7 pp.

AREA 8. APPLES AND OTHER FRUITS - PROCESSING AND PRODUCTS

Problem. Lack of knowledge of the nature and quantities of the various chemical constituents and enzyme systems present in fresh fruits, and of the changes these undergo during processing, is a limiting factor in research designed to develop new and improved products and processing techniques. Knowledge is required on the composition and physical structure of fruits and fruit products, with emphasis on substances responsible for color and flavor, vitamins, and other constituents important in determining consumer acceptance and nutritive value of the products. Composition should be studied in relation to variety, stage of maturity, and environmental conditions of growth; and to changes occurring between harvesting and processing, during processing, and in storage and distribution. Recently developed equipment and techniques have made it possible to isolate, separate, and identify constituents that could not have been handled previously. As basic information is developed, new processing techniques will be applied in the improvement of fruit products, and in more efficient utilization of by-products from fruit processing.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving chemists, biochemists, and chemical engineers engaged in both basic and applied research related to extending the use of fruits in the food processing industries. In the EU program apple products research, and investigations on the chemistry and cell structure of cherries are conducted at Wyndmoor, Pennsylvania. Development of rapidly-reconstitutable dehydrated fruit pieces is also underway at Wyndmoor. Contract research on peaches is in progress at Rutgers University, New Brunswick, on apple texture at the Maryland Agricultural Experiment Station, College Park, and research on the metabolism of red tart cherries was initiated recently at Temple University, Philadelphia.

The Federal (EU) scientific effort devoted to research in this area totals 10.3 professional man-years. Of this total, research on chemical composition and physical properties constitutes 3.7 p.m.-y., including 0.4 p.m.-y. of contract research on apple texture at the Maryland Station and 0.4 p.m.-y. at Temple. Research on new and improved food products amounts to 3.7 p.m.-y., and research on new and improved processing technology amounts to 2.9 p.m.-y., including 0.4 p.m.-y. of contract research on peach processing at Rutgers.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

The States have a continuing and effective program of research on fruit processing and fruit products. Fruits possess unique flavor, color and nutritive qualities which make it desirable although difficult to preserve their unique qualities.

Fruit utilization research begins with evaluation of varieties and selections for processing quality as a service for breeding programs. In addition the relationship of other production and cultural practices to the quality and utilization of the finished products are determined. The biochemical changes associated with post-harvest storage and ripening are studies in an attempt to elucidate the metabolic reactions associated with ripening and to devise means of their control. Respiratory activity of fresh fruits is determined and used as an indication of package environment and to guide package selection. Increased use of mechanical harvesting equipment has been found to directly affect the quality of the fresh fruit and processed products.

Increased interest in pesticide residues on food products has led to the initiation of three regional projects. This regional research is centered around development and evaluation of procedures for reduction or elimination of residues in processed foods. Fruits and fruit products are among the commodities being studied.

The chemical composition and physical properties of certain fruits are being investigated in detail. The color and pigments of fruits are of special interest. Basic research on identification of the polyphenols of fruits and their role in enzymatic browning is continuing. The ultimate texture and reconstitution properties of fruit products are related to the properties of the pectic substances contained in the fruit initially. For example, the polysaccharides of the cell wall and other tissues are under investigation because these compounds are so important to texture of the products. Ethylene metabolism and its role in fruit ripening is under study.

The chemistry of flavor continues to advance with improvements in gas chromatographic procedures. Compounds which have the characteristic properties of fruit flavors are isolated and identified by this technique. Considerable effort is devoted to determining their significance in the flavor response to specific fruit flavors. These findings are correlated with taste evaluations and, through this process, some insight into the development of undesirable flavor is gained.

As previously indicated, study of enzyme mechanisms and properties constitute an important and continuing phase of basic research on fruits. More applied phases of investigation deal with such problems as development of off-flavors in frozen fruit products, enzymatic browning of fresh tissue and methods for control of this form of browning.

Investigations of the ecology, taxonomy and physiology of yeast, molds, and bacteria involved in food fermentations and spoilage are designed to help understand how microbes occur in nature, how they get into foods, and how they bring about either desirable or undesirable changes. This information is used in control of fruit spoilage and in developing and controlling desired fermentation processes such as those involved in wine manufacture. For example, the role of specific microorganisms believed to cause softening of brined cherries is under continuing study. Comprehensive studies deal with the fermentative conversion of fruit juices to wine. These studies

range from fruit composition factors through study of yeast growth factors important in the fermentation to changes in wine during aging. The highly specialized study of the microbiology of olive fermentation continues. Other applied investigations are concerned with evaluation and enumeration of bacteria found in frozen fruit products.

Research directed to development of new or improved fruit products and processing technology continues to be a major part of the fruit utilization program. This work extends from study of the processing quality and suitability of selections and varieties to characterization of the chemical, physical and storage properties of a new fruit product. Basic studies deal with thermal processing requirements and include relationships to mechanism of heat transfer and thermal breakdown of various fruit constituents. Process design including methods, equipment, and plant layout receive study. Newer methods of freezing, dehydrofreezing, freeze-drying, irradiation and dehydration of fruits are under continuing investigation. Effects of chemicals, hydrocooling, refrigerated storage and controlled atmosphere storage and holding are evaluated in terms of the changes in product texture or structure, color and flavor. Product research is designed to provide basic information on product potential and functional properties. Development of processes or products to improve the utilization of fruits involves work on: dehydrated fruits; apple sauce; frozen fruit pies; apple-fruit juice blends; sherry wines; brined cherries; low sugar apple jelly; macadamia nuts; peach concentrates; and grape products. Research on fresh and roasted macadamia nuts has elucidated the quality characteristics of these products and defined optimum conditions for storage.

The total research effort on fruit utilization is approximately 59.1 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties.

1. Chemistry and cell structure of cherries for processing. Post-harvest bruising and aging of red tart cherries causes an increase in the firmness and drained weight of the canned fruit. An attempt was made to determine if there is an actual increase in cellulose content of the bruised and aged fruit. Tissues extracted with cupriethylenediamine (CED), a cellulose solvent, showed no increase in the amount of dissolved cellulose. Microscopic sections treated with CED showed no differences between soft and firm cherries.

2. Factors influencing apple texture. Further information on the chemical composition of apple cell wall constituents was obtained as a result of contract research (Maryland Agricultural Experiment Station). The amount and composition of the hemicellulose fraction was closely related to changes in texture of apples during storage. This fraction consists principally of D-galactose, D-glucose, L-arabinose, D-xylose, and D-galacturonic acid. Glucuronic acid was also present in variable amounts. During maturation and

storage, glucose decreased and glucuronic acid increased to an amount equal to galacturonic acid. Since glucuronic acid interferes with the measurement of galacturonic acid (from pectin hydrolysis) it throws considerable doubt on previous reports of the changes in pectin during storage.

B. New and Improved Food Products.

1. Instant applesauce to be market-tested. A market test is being conducted cooperatively with the Musselman Division of the Pet Milk Company on instant dehydrated applesauce. This product is made by coarsely crushing explosion-puffed dehydrated apple pieces. Approximately 3600 cans of the product, each containing enough material to yield 16 ounces of sauce on reconstitution, were prepared in the Engineering and Development Laboratory.

2. Washington state apples explosion-puffed. Fifty bushels each of Golden and Red Delicious apples grown in Washington state were supplied gratis by the Chelan County Industrial Development Council to determine their suitability for making into dehydrated products employing explosion puffing. Both varieties were shown to be suitable in making explosion-puffed pie slices, instant applesauce, and apple snacks. They were not as well suited, however, to the first two items mentioned as our Eastern grown York Imperials. Snacks made by explosion puffing for use with dry cereals evoked a great deal of interest on the part of potential processors in the Pacific Northwest.

3. New dried cranberry juice. A process developed several years ago in the Engineering and Development Laboratory for dehydrating fruit juices, while retaining their volatile aromas, has now been successfully applied to cranberry juice. The sweetened powder when mixed with water reconstitutes quickly to a sparkling full-flavor drink.

4. Improved apple cider. Diethylpyrocarbonate (DEPC) was effective in reducing the initial microbial count of fresh apple cider. As little as 50 ppm of DEPC destroyed 90 to 99% of the microorganisms in the fresh product. DEPC is more effective in clarified juice than in cloudy juice. Ultraviolet lamps were effective in partially sterilizing fresh cider. However, the color and turbidity of unclarified cider greatly reduced the effective depth of penetration.

C. New and Improved Processing Technology.

1. New puffing gun design. A gun for the explosion puffing of partially dehydrated fruits and vegetables has been designed. Contruction drawings have been provided to industry and one company has already received several orders for commercial units. The new gun employs injected superheated steam in addition to external heat. This results in less heat damage to the product and much higher production rates.

2. Improved essence recovery equipment. A basic engineering study on the fractional distillation of complex mixtures of volatile fruit aromas has provided information for the design of commercial essence recovery equipment

capable of recovering essence components previously lost. This is particularly applicable to the aromas of Concord grape juice. Using the improved equipment design, methylanthranilate, an important aroma constituent, can now for the first time be recovered in good yield.

3. Evaluation of new equipment for cherry processing. Work on the quality of mechanically harvested cherries was continued, by cooperative field studies in Michigan. In connection with the mechanical harvesting of cherries, there are two related developments: mechanical destemmers and electronic sorters. Four types of commercial destemmers were evaluated in 1964. Each type of destemmer effectively removed the major portion of attached stems (87 to 98%) with only minor bruise damage. One available electronic sorting machine effectively eliminated the major defects (decay, limb rub, etc.) but was less efficient in picking out cherries with minor defects (scald, and light color). The capacity of this machine, under a wide range of operating conditions, varied from 742 to 2261 pounds of cherries per hour.

4. Processing characteristics of Eastern peaches. Over 100 new varieties of peaches were evaluated for processing characteristics as a result of contract research (New Jersey Agricultural Experiment Station). Several new varieties of freestones and clingstones were superior in flavor and color to the standard commercial varieties. Two new varieties were unusually high in vitamin A and one was also very high in citamin C.

PUBLICATIONS AND PATENTS -- USDA AND COOPERATIVE PROGRAMS

New and Improved Food Products

Strolle, Eugene O. 1965. Returns essence to dried cranberry juice. Food Engineering 37, 70-72.

New and Improved Processing Technology

Gaston, Harold P., Levin, J. H., and Whittenberger, R. T. 1964. The 2- and 3-layer pallet box makes it possible to handle cherries, peaches and other soft fruit in bulk. Mich. State Hort. Soc. Annual Report 93, 43-45.

Heiland, Wolfgang K., and Eskew, Roderick K. 1965. A new gun for explosive puffing of fruits and vegetables. U. S. Agricultural Research Service, ARS-73-47, 7 pp.

Roger, Norman F., and Turkot, Victor A. 1965. Designing distillation equipment for volatile fruit aromas. Food Technology 19, 69-72.

Whittenberger, R. T., McAdoo, D. J., Gaston, H. P., and Levin, J. H. 1964. Electric sorting machines for tart cherries. Performance studies in 1963. U. S. Agricultural Research Service, ARS-73-45, 8 pp.

Whittenberger, R. T., McAdoo, D. J., Gaston, H. P., and Levin, J. J. 1964. Electronic tart cherry sorters evaluated. Canner/Packer 133, 37.

Harrington, Winfred O. and Hills, Claude H. February 16, 1965. Process for peeling waxy fruit. U. S. Patent 3,169,564.

General

Ogg, C. L. 1965. Report on microchemical methods. J. Assoc. Offic. Agr. Chemists 48, 126.

Steyermark, Al, Alber, H. K., Aluise, V. A., Huffman, E. W. D., Jolley, E.L., Kuck, J. A., Moran, J. J., Ogg, C. L., and Pietri, C. E. 1964. Report on recommended specifications for microchemical apparatus - Oxygen in organic compounds. Microchemical Journal 8, 424-434.

Woodward, Charles F. 1964. Research on apples at the Eastern Regional Research Laboratory. The National Apple News 4, 6-7.

Ogg, Clyde L. September 29, 1964. Apparatus for oxygen-filled closed-flask combustion. U. S. Patent 3,150,932.

AREA 9. TOBACCO - COMPOSITION AND PROCESSING

Problem. Although neither food nor fiber, tobacco nevertheless is grown on about a million acres, and in seven states provided more farm cash receipts than any other field crop in 1964. The farm value is about $1.3 billion. This crop is unique in that it yields about $3.1 billion in Federal and State taxes. Of the problems affecting the tobacco industry, the much publicized charges concerning the effect of tobacco usage on health are the most serious. Although much controversy still surrounds these charges, the importance of the tobacco economy and the seriousness of the charges dictate that research in this area be intensified. Such a program will serve to elucidate more completely the extent of smoking-health relationships and the capabilities of research to alter the observed physiological effects of smoke on animal tissue. Information obtained in such studies may also be of value in other industrial problems, such as the determination of relationships between the chemical composition of tobacco and smoke, and the overall quality of tobacco products. It should be noted that the present program represents a significant reorientation of effort from past endeavor concerned mainly with quality problems.

USDA AND COOPERATIVE PROGRAM

The Department has an expanding program involving many facets of the chemistry and biology of tobacco and its smoke. Much of the work is basic in nature and, although the program is health oriented, many findings of value in industrial problems not related to health may be forthcoming. The present program is divided into five general areas: Basic studies on the composition of cigarette smoke; similar investigations on tobacco leaf; the nature of the pyrolytic products from leaf substances or fractions; the effect of chemical additives on the composition of cigarette smoke; and biomedical studies related to the biological assaying of cigarette smoke.

As a result of a special Congressional appropriation, a substantial part of the present utilization research program will be performed at the University of Kentucky, Lexington, Kentucky, under several contracts and a cooperative agreement.

The Federal work is conducted at Wyndmoor, Pennsylvania, Linwood, Pennsylvania, Durham, North Carolina, and Lexington, Kentucky, and a total of 27.5 professional man-years is involved. In composition studies on smoke, including the development of improved analytical methods, 7.3 p.m.-y. are involved at Wyndmoor, Pennsylvania, and 3.1 p.m.-y. are under contract at the University of Kentucky. In similar studies on leaf, 3.0 p.m.-y. are involved at Wyndmoor, Pennsylvania, on a study of oxidation products of leaf and 2.0 p.m.-y. are under contract at the Research Triangle Institute, Durham, North Carolina for a study of the neutral resins of leaf. In studies on cigarette additives, 1.7 p.m.-y. are under contract at the Houdry Process and Chemical Company, Linwood, Pennsylvania, on the development of special additives and

1.0 p.m.-y. of Eastern Division personnel is involved at Lexington, Kentucky, under cooperative agreement with the University of Kentucky on the evaluation of the special additives and the development of other cigarette modifiers. Studies on pyrolytic products are being pursued with 1.2 p.m.-y. under contract at the University of Kentucky. A total of 8.2 p.m.-y. are involved under contracts at the University of Kentucky on bioassay studies. In addition, the Cigar Manufacturers' Association of America supports a research program on cigar smoke at Wyndmoor that is the equivalent of 2.0 p.m.-y.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

A modest program of research related to the utilization of tobacco is conducted by the stations. A number of studies on tobacco plant metabolism have been related to enzyme reactions and chemical intermediates produced during growth. Progress is being made toward identification of more than 150 compounds contained in the aromatic oil fraction of flue-cured tobacco. Development of analytical methods using gas chromatography and infrared spectra has contributed to this work. Formation of hydrocarbons in the leaf wax, photosynthetic production of glycolic acid in seedlings, and enzymic degradation of fatty acids during seed germination illustrate the variety of station research approaches to tobacco plant chemistry.

Compounds of great importance to tobacco curing are under investigation both as to source during growth and to fate during post-harvest processing. Plant tissue conversion of amino acids into polyphenols has been demonstrated, and the rule of oxidases to further catalyze the oxidation of polyphenols is continuing to unfold knowledge on the mechanisms of curing and aroma development. Investigations of machines to mechanize tobacco culture, harvest and curing are continuing. Basic engineering data on specific heat transfer characteristics of tobacco leaves have been determined for use in curing studies and have been applied as well to work on plastic mulches for weed control in seed beds. Study of agronomic practices is continuing. Control of sucker formation with herbicides such as maleic hydrazide is being evaluated. Nitrate nitrogen fertilizers have been reported important in upping U. S. type 41 cigar tobacco yields.

Market survey studies of U. S. cigarette brands is continuing to show that the percentages of different tobacco types remains virtually unchanged from year to year. Of the tobacco leaf components, flue-cured and oriental types increased only slightly. Usage of tobacco sheet (homogenized) has tended to increase and burley to decrease somewhat.

Fermentation studies on cigar tobacco are underway at Puerto Rico and other stations. Controlled modification of tobacco aroma is being explored via biochemical genetic approaches.

The tobacco program at the state stations involves 8.6 p.m.-y.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Composition of Tobacco Smoke.

1. Cigarette smoke. A study was made of certain of the published methods for determining benzo(a)pyrine in cigarette smoke. This compound is of major concern because of its potent cancer-producing properties. Of the methods studied, all were inadequate in respect to reproducibility and time required for the analysis. An improved method was then developed which gave more consistent results and reduced the analytical time by one-third. However, further improvement is necessary and work is continuing.

In work on the composition of cigarette smoke, crude fractionation of smoke condensate from 50,000 cigarettes showed that 25-30% of the condensate is water-soluble. Attempts to separate the water-soluble fraction by Sephadex gel filtration were unsuccessful, probably because a wide range of molecular weight substances is present. Comparative separations of the non-polar, water-insoluble neutrals of smoke showed that silicic acid is generally superior to alumina as an adsorbent for the initial stages of separation. The presence of a large number of olefinic structures, including neophytadiene, in the hydrocarbon fraction was confirmed. During the course of some of these separations, a study was made of the substances which codistill with ether from an ether-soluble, neutral fraction of smoke. At least five hydrocarbons (dipentene, styrene, o-xylene, toluene, and ethylbenzene), four carbonyls (acetaldehyde, isovaleraldehyde, α-methylbutyraldehyde, and n-propyl methyl ketone) and one ester (ethyl acetate) were tentatively identified. Of these, only α-methylbutyraldehyde was previously unreported in smoke.

It is anticipated that work on the composition of heterocyclic bases of smoke at the University of Kentucky will be initiated early in fiscal year 1966.

2. Cigar smoke. The hydrocarbons of cigar smoke were investigated extensively. Qualitative and quantitative similarities were observed between these findings and those previously published on cigarette smoke hydrocarbons. The following aromatic and related hydrocarbons were found in cigar smoke: benzene; toluene; ethylbenzene; m- and p-xylene; o-xylene and/or styrene; 1,2,4-trimethylbenzene; dipentene; and m- and/or p-ethyltoluene. Of these, the ethyltoluenes were previously unreported in tobacco smoke. Also, a series of aliphatic paraffins ranging from C_{13} to C_{25} were found in cigar smoke; these components have been reported previously in cigarette smoke.

B. Composition of Tobacco Leaf.

1. Oxidation products and related substances. Work has continued on the dark pigments of leaf which may be responsible for leaf color. Sephadex gel filtration has separated the whole pigment into a number of fractions with widely varying molecular weights. Of the fractions studied, all contained rutin, chlorogenic acid, and 20 amino acids, and some contained iron. Sugar analyses of hydrolysis mixtures indicated about 0.5 - 1.0 mole of rutin per 1300 g. of pigment. Attempts to obtain further information based on quinic acid

determinations and on separation of methylation products have failed thus far. Pyrolysis of the pigments has produced some interesting results (see below).

2. Neutral resins. Pilot experiments have been performed on the composition of the hexane-extractable neutral resins of leaf. The higher molecular weight substances showing carbonyl groups in the infrared spectra appear to be mainly esters which consist of acids having unsaturation but no aromaticity. Carbonyl compounds appear to be present in relatively small amounts and do not react readily with Girard's reagent. For the first time, a technique for separating non-polar substances by molecular weight ("gel permeation") was used, and two major groups of substances were isolated from the hexane and acetone solubles of leaf. The groups had average molecular weights of 723 and 1275, respectively. This technique promises to be of great value in separating lower molecular weight, known leaf components from the high molecular weight resins.

3. Volatile constituents. Final work on relationships between chemical composition and the organoleptic properties of leaf was completed. A system was developed for collecting the volatile substances of leaf which are emitted during leaf storage in a closed container. At least 35 components were found in the volatile mixture, of which tentative identifications were obtained on 17; included in the 17 were the following compounds previously unreported in tobacco leaf: n-pentane, n-hexane, n-heptane, benzene, toluene, three isomeric xylenes, n-caproaldehyde and methyl propionate.

C. Pyrolysis Studies.

The polyphenol-amino acid pigment discussed under Oxidation Products above was pyrolyzed at a temperature approximating that of a burning cigarette, i.e., 880°C. Most of the products were gases not condensable in dry ice traps. Evidence was obtained for the presence of at least 16 polynuclear aromatic compounds in the nonvolatile residue, including benzo(a)pyrene and dibenz(a,h)anthracene, both of which are potent cancer-producing substances known to be present in cigarette smoke. The yields of polynuclears appear to be quite high, e.g., about 10 milligrams of benzo(a)pyrene from 10 grams of pigment.

It is anticipated that pyrolysis studies to be conducted at the University of Kentucky will be initiated early in fiscal year 1966.

D. Cigarette Additives.

A system for measuring the coal temperature of cigarettes was developed. Values of about 875°C. were obtained which are comparable with the more reliable literature values. The claimed depression of the normal cigarette coal temperature by 50° - 100° C. using a type of hydrated alumina in cigarettes was confirmed. The effect of altering the pH of tobacco by adding citric acid or lime to cigarettes was studied. Citric acid imparts a light, mild flavor to the smoke; lime does not produce any striking changes in smoke flavor. With both citric acid and lime, an apparent increase occurred in the

benzo(a) pyrene content of the smoke compared to cigarettes without additives. However, the precision of the analytical method was not entirely satisfactory, and confirmation of this finding is needed.

E. Bioassay Studies.

It is anticipated that technical work in this area at the University of Kentucky will be initiated early in fiscal year 1966.

PUBLICATIONS AND PATENTS -- USDA AND COOPERATIVE PROGRAMS

Composition of Tobacco Smoke

Burdick, D., Chamberlain, W. J., and Stedman, R. L. 1964. Composition studies on tobacco. XIX. Steam-volatile neutral substances in smoke of cigarettes having different organoleptic properties. Tobacco Science 8, 82-85.

Osman, Stanley and Barson, J. 1964. The volatile bases of cigar smoke. Phytochemistry 3, 587-590.

Osman, S. and Barson, J. 1964. Hydrocarbons of cigar smoke. Tobacco Science 8, 158-160.

Schmeltz, Irwin, Stedman, R. L., Chamberlain, W. J., and Burdick, D. 1964. Composition studies on tobacco. XX. Bases of cigarette smoke. J. Sci. Food Agr. 15, 774-781.

Schmeltz, Irwin, Stedman, R. L., and Chamberlain, W. J. 1964. Improved method for the determination of benzo(a)pyrene in cigarette smoke condensate. Anal. Chem. 36, 2499-2500.

Stedman, R. L., Burdick, D., Chamberlain, W. J., and Schmeltz, I. 1964. Composition studies on tobacco. XVIII. Steam-volatile acids in smoke of cigarettes having different organoleptic properties. Tobacco Science 8, 79-81.

Stedman, R. L. and Stills, C. D. 1964. The flavor contribution of Turkish tobacco in blended cigarettes. U. S. Agricultural Research Service, ARS-73-43, 8 pp.

Stedman, Russell L. and Stills, Charles D. April 27, 1965. Turkish tobacco substitute for smoking tobacco. U. S. Patent 3,180,340.

General

Filipic, V. J. and Burdick, D. 1964. Polyethylene microcells for infrared analysis. Appl. Spectroscopy 18, 65-66.

Ogg, C. L. 1964. Determination of particulate matter and alkaloids (as nicotine) in cigarette smoke. J. Assoc. Offic. Agr. Chemists 47, 356-362.

Ogg, C. L. 1965. Report on tobacco. J. Assoc. Offic. Agr. Chemists 48, 105.

AREA 10. MAPLE SAP AND SIRUP - PROCESSING AND PRODUCTS

Problem. The extensive unused stands of sugar maple trees are largely located in agriculturally depressed areas that are commonly devoted to small-scale dairy farming. Since only a small percent of the available sugar maple trees are presently tapped for sap production, and about 50% of the sirup consumed in the United States is imported, untapped sugar maples represent a good potential source of increased cash income for farmers in these areas. The maple area includes 14 states from Minnesota to Maine and south to Virginia. Under proper conditions, maple sirup can be a 6-weeks seasonal crop not in competition with other farm activities and with a per acre value equal to or exceeding that of other farm products. Based largely on recent research carried out in the Department and the State Experiment Stations, the methods of collecting and processing sap into sirup are being streamlined. This has resulted in greatly increased efficiency and larger hourly returns to the sirup producer for his labor. The advent of tube collection and transportation of sap has reduced the cost of sap handling 40% and has eliminated much hand labor. Oil-firing of evaporators and improved systems of steam removal have provided efficient and sanitary plants. The taphole germicidal pellets and sanitary methods of sap handling have tended to stabilize crop yields and standardize sirup quality. While the results of previous research have contributed to modernization of the industry, much more information is needed so that all operations for the production of high-quality maple sirup and other maple products can be conducted in a predictable, efficient manner. Not only can the low income farms be greatly benefited, but the existing maple industry can be put on a higher economic plane and modernized to be made competitive with other crop and livestock farming to bring about improved land use.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving chemists, biochemists and microbiologists. These scientists are engaged in both basic and applied research in investigations concerned with the problems of improving sap handling and processing, producing high-quality maple sirup, and developing new outlets for all maple products while lowering the cost of the product. Most of this work is conducted at Wyndmoor, Pennsylvania.

The Federal scientific effort devoted to research in this area totals 3.2 p.m.-y. Of this number 1.0 are devoted to study of the chemical composition and physical properties of maple sap and sirup, 1.0 to microbiology of maple products and 1.2 to new and improved food products and processing technology, including 0.2 p.m.-y. in contract research on sap storage, with J. L. Sipple & Son, Bainbridge, New York. In the research work cooperation is maintained with personnel of the Federal Extension Service in maple-producing states and with Cornell University.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

Research on maple sap, sirup and products involves four projects in three states including Pennsylvania, New York and Vermont. Investigations are currently concerned with central evaporator processing and its economic feasibility; study of sap flow and water movement in trees; modern sap collecting systems; and marketing systems and the potential demand for maple products.

The market potential and demand study has indicated that the U. S. is currently importing more maple products from Canada than U. S. production; that 50% of total U. S. production is sold retail by maple producers; and that 13% of the total is sold wholesale in consumer packages and 37% wholesale in drum containers. Additional research will examine the relationships of scale of production, advertising, sugarhouse location, time of sales, population density, production levels, and income levels.

The number of professional man-years devoted to maple products utilization is 0.8.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

Investigations on maple sap and sirup to obtain information on which to base improved techniques for making better maple products at lower cost are being carried out in three different areas: (1) composition of maple flavor, (2) fermentation studies and (3) improvement in processing and products.

A. Composition Studies.

1. Composition of maple flavor. The chemical nature of the maple flavor constituents that have been identified so far (vanillin, coumarin, syringaldehyde, coniferyl aldehyde, dihydroconiferyl alcohol, 2,6-dimethoxybenzoquinone, acetol, and cyclotene) suggests their possible formation from soluble lignins and from combinations with sugar break-down products. The data being obtained on the nature and source of maple flavor are of utmost importance in the development of improved processing methods and new maple products. Positive identification of sap-contained flavor precursors should be invaluable in selection of maple trees for propagation.

B. Fermentation Studies.

1. Reclaiming buddy sap. Buddy sap produced when the tree comes out of dormancy yields an unmarketable sirup because of the unpalatable flavor. The use of the germicidal pellets tends to favor buddy sap production since it extends sap flow later into the spring. Removal of the buddy principle from sap by fermentation was successfully conducted on a commercial scale this year.

2. Ultraviolet sterilization of sap. The use of ultraviolet radiation for controlling microbial growth was investigated to furnish data essential for sap storage studies. This physical method of sterilization was highly

effective even on rapidly flowing sap. No detectable effects on the flavor or color of the sirup made from the treated sap were noted. This process eliminates the objectionable features of chemical preservatives.

C. Processing and Products.

1. New products. A new continuous process has been developed for the high-flavoring of maple sirup. This process permits utilization of the top grades of maple sirup (80% of U. S. production) for making blended (cane-maple) table sirup. Formerly, these blending sirups were a major import from Canada.

2. Central sap evaporation plants. Utilization of a cooperative-owned vegetable canning plant, normally idle during the maple sap season, as a central sap evaporation plant was successfully conducted on a pilot plant scale. These plants, because of the large capacityof their facilities, can (a) provide an outlet for sap produced in a large area and (b) lower sirup processing costs which should result in larger markets because of lower prices to the consumer.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

Filipic, V. J. and Underwood, J. C. 1964. Composition of maple sap and sirup. Some aromatic compounds in sap. J. Food Sci. 29, 464-468.

Underwood, J. C. 1964. Collaborative study of the determination of formaldehyde in maple sirup. J. Assoc. Offic. Agr. Chemists 47, 548-550.

Underwood, J. C. and Filipic, V. J. 1964. Source of aromatic compounds in maple sirup flavor. J. Food Sci. 29, 814-818.

Willits, C. O. 1965. Report on methods of analyses for maple products. J. Assoc. Offic. Agr. Chemists 48, 144.

Willits, C. O. 1965. Report of liaison with committee E15 of the American Society for Testing and Materials. J. Assoc. Offic. Agr. Chemists 48, 224.

Fermentation Studies

Kissinger, J. C., Sipple, L., and Willits, C. O. 1964. Maple sap delivered to a central evaporator plant -- a progress report. Natl. Maple Syrup Digest 3, 8-10.

Wasserman, A. E. 1964. Yellow pigment production by Pseudomonas geniculata in maple sap. Appl. Microbiol. 12, 287-288.

Wasserman, Aaron E., and Willits, C. O. 1964. Maple sirup. XXII. Controlled fermentation of buddy maple sirup. Food Technol. 18, 1966-1968.

New and Improved Food Products and Processing Technology

Robbins, Putnam W. 1965. Influence of tapping techniques on maple sap yields. Mich. Agr. Expt. Sta. Res. Report 28, 3 pp.

Sipple, Lloyd, Winch, Fred, and Willits, C. O. 1964. Multiple evaporator installations. Natl. Maple Syrup Digest 3, 6-8.

Willits, C. O. and Sipple, Lloyd. 1964. Facilities for the manufacture of maple confections. Natl. Maple Syrup Digest 3, 10-12.

AREA 11 - HONEY - PROCESSING AND PRODUCTS

Problem. Essential pollination of over fifty crops depends almost completely on the honeybee because of changes in agricultural practices over recent years. The importance of the beekeeper in our economy is thus emphasized. As most of his income results from the sale of honey, the beekeeper not only is subject to uncertainties of crop and weather, but must also contend with disease, losses of crop and bees from insecticides, rising costs of needed equipment and materials, lack of trained help, all compounded with uncertain and depressed markets for honey. Because of the relatively small size of operations and the scattered nature of the industry, the honey producer is out-researched, out-promoted and out-advertised by competing sweetening agents. Improved processing methods and equipment, better control of product quality, outlets for lower-grade honey, stable export markets, increased use of honey, both in food manufacture and the home, and increased industrial use of byproducts are all needed to provide an expanding market and encourage the beekeeping industry.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long term program involving chemists and biochemists engaged in both basic studies and the application of known methods and principles to the solution of honey producers' problems. This work is done at Wyndmoor, Pennsylvania.

The Federal scientific effort devoted to research in this area totals 4.0 professional man-years, all devoted to research on enzymes in honey.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

Four stations are conducting research on honey utilization under four projects. Previous research involving study of the chemical and physical properties of California honeys has been completed.

Work on the design and evaluation of honey processing equipment continues with current effort being focused upon testing flash heating, cooling and bottling equipment. Related research at the Wisconsin station is directed to study of the use of electric, automatically controlled honey and wax separators, and heaters and coolers for handling honey.

Efforts to identify the special ingredients of honey and the cause(s) of either desirable or undesirable flavor continues. New research has been initiated to determine the relationship of pesticide residues to honey quality and marketability.

Total research effort on honey is approximately 1.3 p.m.-y.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Enzymes.

The importance of a healthy export market in honey cannot be overestimated. In addition to sharp price competition, American exporters must meet restrictive requirements in principal import markets with respect to so-called quality factors in honey. The availability of complete information on such honey components as enzymes, inhibine (antibacterial factor), and hydroxymethylfurfural (HMF) may be most useful to exporters, as well as means for determining these factors. Studies on the properties and characterization of the enzymes and protein materials of honey are continuing, with application of modern methods of laboratory evaluation unavailable a few years ago. The determination of the protein content of honey, both as done for many years and as proposed in a new procedure, gives erroneous results; a suitable method has been developed. Also recently developed was a highly-simplified, field method to permit the honey packer to assay for diastase in the packing plant to allow intelligent selection of batches of honey for export. Basic studies on honey enzymes were continued. The glucose oxidase of honey, which is responsible for its antibacterial activity was found to have an unusually high optimal substrate concentration (2.7 molar). The invertase of honey has been found to consist of a number of very closely-related constituents, much more difficult to separate than the similar instances reported for other enzymes. Evidence for this is now at hand from both gel electrophoresis and ion-exchange cellulose chromatography.

It is necessary to foresee coming changes in quality requirements of European importers. As an example, when several interests in Europe proposed inhibine content as a quality factor, we already had research information on the nature of inhibine and its occurrence and stability under processing conditions. This enabled us to point out its unsuitability as a quality factor for honey.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Enzymes

Schepartz, Abner I. 1965. The glucose oxidase of honey. II. Stereochemical substrate specificity. Biochim. Biophys. Acta 96, 334-336.

White, Jonathan W. Jr., and Subers, Mary H. 1964. Studies on honey inhibine. 3. Effect of heat. J. Apic. Res. 3, 45-50.

White, Jonathan W. Jr. and Subers, Mary H. 1964. Studies on honey inhibine. 4. Destruction of the peroxide accumulation system by light. J. Food Sci. 29, 819-828.

General

White, Jonathan W. Jr., Kushnir, Irene, and Subers, Mary H. 1964. Effect of storage and processing temperatures on honey quality. Food Tech. 18, 555-558.

White, Jonathan W. Jr., Subers, Mary H. and Kushnir, Irene. 1964. How processing and storage affect honey quality. Apic. in W. Australia 1,8-12. (Reprinted from Gleanings in Bee Culture 91, 422-425.

AREA 12 - REPLACEMENT CROPS - UTILIZATION POTENTIAL - EASTERN REGION

Problem. Farmers could achieve economic use of their land if new and profitable crops were available that would have different end-uses than crops presently grown. For example, it would be advantageous to develop a new oilseed crop yielding unique fatty acids that could find industrial use in applications for which acids from presently available domestic oilseed crops are unsuitable.

To develop a new crop, three basic steps are involved: (1) survey of wild plants, in cooperation with plant scientists, to identify those having both potentially valuable components and promising agronomic potential for use in the U. S.; (2) detailed physical and chemical characterization of components and basic research to obtain clues to likely end-uses; (3) selection of the most promising species, followed by additional utilization research to explore uses and demonstrate industrial potential and by additional agronomic research to establish proper cultural practices and select the best strains and varieties.

Only after these steps have been successfully accomplished can a proposed new crop be offered to agriculture and industry for introduction and development. Obviously, a program of this type is a long-range one. Yet such long-range research is necessary if agriculture and the nation are to benefit from availability of the best practical crop plants.

To achieve this objective, survey and characterization work needs to be continued, since the greater the number of species examined, the greater will be the opportunities for finding plants meeting the criteria of high utilization and agronomic potential. Work of the Department has already revealed several promising sources of new potentially valuable water-soluble gums, pulp fibers, and oils containing unique fatty acids such as hydroxy unsaturated acids, capric acid, epoxy acids, and unusual long-chain fatty acids. In order to demonstrate the potential of these new materials, further work is required on their physical and chemical properties and reactions, on processing to obtain maximum recovery from source plants and on byproducts from processing, such as oilseed meals.

USDA AND COOPERATIVE PROGRAM

Work on new crops was terminated June 30, 1965. In F.Y. 1965 this work totaled 4.3 professional man-years, and was concerned with a study of the oil obtained from the seed of an ironweed (Vernonia anthelmintica) of Indian and Pakastani origin and also recently (beginning in December 1964) with the seed oil from Euphorbia lagascae, an introduction from Spain. These studies have been in cooperation with the Northern Utilization Research and Development Division, the Crops Research Division, and the Western Utilization and & Res.

Development Division. These oils contain epoxy fatty acids, potentially useful industrial chemicals.

PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

Discovery and preservation of valuable plant germ plasm is a continuing objective of the station program in new crops. Much of the research in this area is being done via four regional projects and in cooperation with regional centers. A large portion of the work is cooperative with USDA. Each year many plant introductions are grown and evaluated. Annual and perennial crops possessing potential for industrial or agricultural use are further evaluated for agronomic and chemical qualities. These include crops for paper pulp, pigments, drugs, tannins, essential oils, insecticides, polysaccharide gums, and oils rich in acids of unusual structure. Assay of native and introduced tropical plants for products of economic value receives special attention. New varieties of fruits, vegetables, and grasses better resistant to disease and drought are continually sought.

Basic aspects of this program involve study of the biochemical and physiological basis for differences in crop plants. Attempts are made to determine if differences in biochemical or physiological processes can be associated with particular factors related to quality. Information concerning carbohydrate transformations is sought through study of carbohydrate formation and enzyme mechanisms. Horticultural specialty crops are gaining in importance. A number of studies are underway to facilitate rapid development of this industry.

The total scientific effort devoted to replacement crops is 8.4 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

Vernonia seed oil and trivernolin obtained by commercial extraction of imported and domestic Vernonia seed under our supervision was refined and more than thirty industrial requests for samples were fulfilled. Several large manufacturers of plasticizers evaluated these products and reported favorably on their use in poly(vinyl chloride) (PVC) formulations. One report stated "both materials proved to be very interesting as stabilizing plasticizers for PVC, particularly the trivernolin oil. Trivernolin compares favorably with epoxidized soybean oil for all permanence properties and light stability and shows slight improvement over Peroxidol 780 for long-term heat stability. Both trivernolin and the Vernonia seed oil have low temperature properties which would be comparable to an epoxidized tallate ester such as Peroxidol 781."

Over three hundred samples of seeds from the 1964 crop, grown in collaboration with the New Crops Research Branch, CRD, ARS, were analyzed (bringing the total number to about 800 samples covering a nationwide area) for quality as judged by oil content and oxirane oxygen and free fatty acid content of the seed oil. Analytical methods were scaled down ten to one in order to analyze and save seed for individual plants selected through cooperation with

Purdue University investigators who are performing breeding experiments to obtain high quality, early maturing seed.

A series of aliphatic esters of vernolic acid was prepared for evaluation as primary plasticizers for PVC. Preliminary results have indicated that these esters have potential use as low temperature plasticizers.

A study of the composition of the unsaponifiable fraction which amounts to six to eight percent of the composition of Vernonia anthelmintica seed oil has been started. Infrared spectra and chemical tests indicate that the unsaponifiables contain a number of individual sterols that collectively account for about fifty-eight percent of this fraction. Two hydrocarbon portions representing about four to five percent of the unsaponifiables were isolated by column chromatography. The larger of the two portions was a saturated hydrocarbon with a chain length of about C 20.

Preliminary work was done with the oilseed, Euphorbia lagascae. This seed contains about fifty percent oil rich in cis-12,13-epoxy-cis-9-octadecanoic (vernolic) acid (sixty to seventy percent). The vernolic acid seems to have a random or restricted random distribution in the glycerides of this species. Prepressing experiments indicate that a prime high quality light-colored oil can be obtained in a good yield from this seed and additional good quality oil can then be extracted from the pressed seed cake. The enzyme systems of Euphorbia lagascae are not as active as those present in Vernonia anthelmintica seed and do not have the unique characteristics of the latter.

Summary of Five Years' Research On Epoxy-bearing New Crop Seed Oils.

Since this work on new crop seed oils was discontinued at the Eastern Division, it seems appropriate to give a brief resume of the overall progress made at the Eastern Division from May 19, 1960, to May 19, 1965.

Four major components, trivernolin, 1,3-divernolin, vernolic acid and 12,13-dihydroxyoleic acid and an unsaponifiable fraction were isolated and prepared in quantity from this seed oil. Foreign and domestic Vernonia seed varied in oil content within wide limits, (10% to 32%; good quality seeds provided 25% to 30%, with 70% to 75% trivernolin content); the relative amounts of components present in the oil depended upon the manner in which the seed was handled in processing. Minor components found in the seed oil as glycerides were linoleic acid, about 9%; oleic acid, about 2%; palmitic, about 3%; stearic, about 1%; and other fatty acids in trace amounts.

Optimum conditions were worked out for processing Vernonia seed to trivernolin-rich oil and directly to trivernolin, chief component in the oil. These conditions were tested on a larger scale at EU, in a commercial pilot plant and in a small soybean extraction plant under EU investigators' supervision. Vernonia seed was processed in the soybean plant without modification of existing equipment and without lipolysis.

Industrial testing and evaluation studies performed at EU confirm the value of Vernonia products as plasticizer-stabilizers in poly(vinyl chloride) formulations. The Vernonia seed oil and trivernolin compared favorably with epoxidized soybean oil and the metal salts of vernolic acid were superior as heat and light stabilizers of PVC to those presently in use.

The enzyme systems present in Vernonia seed were found to be unique; inhibition and acceleration techniques for their control were developed. A lipolytic principle specific for the hydration of the number two position of glycerides appeared to be present. An enzymatic method for conversion of vernolic acid to 12,13-dihydroxyoleic acid was discovered.

With respect to refining and improving the quality of Vernonia products, (1) poor quality domestic Vernonia seed was upgraded by a process of air-flotation which separated a light fraction (immature seed high in dihydroxyoleic acid) from a heavy fraction (high in the desired epoxyoleic acid); (2) Vernonia oil was refined to high quality for evaluation studies by a combination of adsorbents and removal of unsaponifiables (about 10%) at low temperatures; (3) highly purified (about 100%) trivernolin, divernolin and vernolic acid were prepared by a combination of procedures, crystallization, treatment with adsorbents, solvent partition and column chromatography.

Stability tests were completed on Vernonia seed, the seed oil and on trivernolin. Storage of whole seed for periods up to three years did not affect the quality of the seed oil but the ability of the enzyme systems to act when the seed was crushed appeared to increase with time. The epoxy content of low free fatty acid content Vernonia oil and trivernolin changed only slightly when these products were stored at room temperature for six months. However, the viscosity of the samples that were exposed to light increased greatly, indicating changes in the physical nature of these products.

Nutritive value studies on Vernonia seed meal in cooperation with the Pharmacology Laboratory, WU, have demonstrated that in rat feeding Vernonia seed pericarp and kernel when autoclaved will produce good growth rates at twenty percent dietary levels compared with rates of control animals. Pancreatic enlargement and growth inhibition were observed in rats fed unautoclaved kernel fraction from Vernonia meal. The nature of this undesirable factor which was destroyed by heat is not known. When fed to rats Vernonia seed oil and trivernolin were purgatives.

PUBLICATIONS AND PATENTS -- USDA AND COOPERATIVE PROGRAMS

Utilization of Oilseeds Containing Epoxidized Oils

Herb, S. F., Magidman, P., and Barford, R. A. 1964. A satisfactory GLC column for the determination of epoxyoleic acid in seed oils. J. Am. Oil Chemists Soc. 41, 222-224.

Krewson, C. F., and Scott, W. E. 1964. Vernonia anthelmintica (L.) Willd. Extraction of oil and trivernolin from the seed. J. Am. Oil Chemists Soc. 41, 422-426.

Riser, G. R., Bloom, F. W., and Witnauer, L. P. 1964. Evaluation of butyl stearate, butyl oleate, butyl ricinoleate and methyl linoleate as poly-(vinyl chloride) plasticizers. J. Am. Oil Chemists Soc. 41, 172-174.

Scott, W. E., and Krewson, C. F. 1965. Vernonia anthelmintica (L.) Willd. The effect of storage on the epoxy content of the seed oil and trivernolin. J. Am. Oil Chemists Soc. 42, 147-149.

Krewson, C. F., Ogg, C. L., Oelshlegel, F. J., Jr., Hale, Reginald, and Hale, A. H. 1965. Processing ironweed (Vernonia anthelmintica)seed in a soybean extraction plant. J. Am. Oil Chemists Soc. 42, 563-565.

White, G. A., and Haun, J. R. 1964. Vernonia Research Summary, 1963. CR-30-64. U. S. Dept. of Agriculture Crops Research Division, Beltsville, Md.

Krewson, C. F., Scott, W. E., and Ard, J. S. November 17, 1964. Process for obtaining trivernolin. U. S. Patent 3,157,676.

Krewson, C. F., and Ard, J. S. January 12, 1965. Process for isolation of divernolin and trivernolin. U. S. Patent 3,165,540.

Steroidal Sapogenins

Preston, W. H., Jr., Haun, J. R., Garvin, J. W., and Daum, R. J. 1964. Several aspects of growth, development and sapogenin yield of tubers of Dioscorea spiculiflora. Econ. Bot. 18, 323-328.

LINE PROJECT CHECK LIST -- REPORTING YEAR 64 to 65

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
E6 2	Milk Products Utilization Investigations. Program Leadership	Wyndmoor, Pa. and Washington, D.C.		
E6 2-81(Rev.)*	Removal of radioactive contamination from milk	Beltsville, Md.	No	
E6 2-83 (C)*	Chemistry of Cheddar cheese flavor	Columbus, Ohio	Yes	1,2-D-2
E6 2-84	A laboratory and pilot plant study of the effect of chemical additives on the storage stability of evaporated milk	Washington, D.C.	Yes	1,2-B-3
E6 2-85 (Rev.)	Interactions of milk proteins in solution	Wyndmoor, Pa.	Yes	1,2-C-2
E6 2-87	Methods for making fat-free and low-fat cheese	Washington, D.C.	Yes	1,2-D-2
E6 2-88	Physico-chemical studies of factors influencing milk fat-plasma emulsion stability	Washington, D.C.	Yes	1,2-C-2
E6 2-89 (C)*	Development of improved techniques for evaluating importance of flavors in new concentrated milk products	Corvallis, Oregon	Yes	1,2-A
E6 2-90*	Improvement of concentrated whole milk products	Washington, D.C.	Yes	1,2-B-3
E6 2-92*	Development of increased food outlets for nonfat milk solids	Washington, D.C.	Yes	1,2-D-3
E6 2-93	Development of a commercially feasible process for preparing a beverage quality dry whole milk having adequate shelf life	Wyndmoor, Pa.	Yes	1,2-B-1
E6 2-94 (C)	Effects of nonfat dry milk on bread yeast fermentation	Madison, Wis.	Yes	1,2-D-3
E6 2-95 (C)	Increased protein stability of evaporated milk: study of calcium phosphate-casein micelles	Columbus, Ohio	Yes	1,2-B-3
E6 2-96	Improving the flavor stability of anhydrous milk fat	Washington,D.C.	Yes	1,2-D-1
E6 2-97	The chemistry of bacterial spores	Washington,D.C.	Yes	1,2-C-4
E6 2-98	Studies on stale flavor in sterile milk and development of means to prevent its formation	Washington,D.C.	Yes	1,2-A
E6 2-99	Improved sterile whole milk concentrates: the production of reversible sol-gel transformations in high solids sterile concentrates	Washington,D.C.	Yes	1,2-B-3
E6 2-100 (C)	Removal of radioactive strontium from milk on a commercial scale	Springfield, Mo.	Yes	1,2-E
E6 2-101	Ribosomal nucleic acids	Wyndmoor, Pa.	Yes	1,2-C-2
E6 2-102 (C)	Heat stability of individual milks	St. Paul, Minn.	Yes	1,2-C-2
E6 2-103	Enzyme studies relating to milk	Wyndmoor, Pa.	Yes	1,2-C-1
E6 2-104	Casein properties	Wyndmoor, Pa.	Yes	1,2-C-1
E6 2-105 (Gr)	Physical changes in milk and milk concentrates associated with steam injection and bubble collapse	Raleigh, N.C.	Yes	1,2-B-3
E6 2-107 (C)	Relation of milk fat composition, particularly fat and protein, to dietary of cow	College Park, Md.	Yes	1,2-D-1
E6 2-108 (Gr)	Flavors and their precursors in milk derived from pasture or dry feeding practices	College Park, Md.	No	
E6 2-109 (Gr)	Study on the desirable flavors of butter: Isolation and identification of specific flavor contributing compounds and their precursors	Corvallis, Oregon	Yes	1,2-A
E6 2-111 (Gr)	Lactones, methyl ketones and their precursors in milk products: effects on off-flavors and development of procedures for their control	University Park, Pa.	No	
E6 2-113	Improvement of dried whole milk	Washington, D.C.	Yes	1,2-B-2
E6 2-115**	New and improved processing equipment	Washington, D.C.	Yes	1,2-D-4

LINE PROJECT CHECK LIST -- REPORTING YEAR 64 to 65

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area and Subheading
UR-A7-(60)-5	Milk coagulating enzymes	India	Yes	1,2-D-2
UR-A7-(60)-11	Sulfur compounds in relation to flavor and stability of milk	India	Yes	1,2-D-2
UR-A7-(60)-13	Export outlet for nonfat dry milk as additive to buffalo milk in cheese manufacturing	India	Yes	
UR-A7-(60)-16	Phosphoproteins of milk	India	No	
UR-A7-(60)-22	Proteose-peptone fraction of milk	India	Yes	1,2-C-2
UR-A7-(60)-48 **	Dipicolinic acid synthesis in bacterial spores	India	No	
UR-E8-(60)-1*	Growth-promoting factors for lactic acid bacteria	Finland	Yes	1,2-A
UR-E8-(60)-16	Dietary factors controlling flavor in milk	Finland	Yes	
UR-E9-(60)-46	Nonprotein nitrogenous constituents of milk	France	No	1,2-A
UR-E9-(60)-47	Proteolytic activity of rennin on casein	France	Yes	1,2-C-2
UR-E9-(10,60)-80	Structure of nucleic acids	France	Yes	1,2-C-3
UR-E10-(60)-3	Surface changes in fat globules of dried whole milk	West Germany	Yes	1,2-B-2
UR-E21-(60)-7	Increasing vitamin B in cheese	Poland	No	
UR-E21-(60)-21	Mechanisms of cheese ripening process	Poland	Yes	1,2-D-2
UR-E25-(60)-18*	Protein destabilization in frozen milk	Spain	Yes	1,2-B-3
UR-E25-(60)-37**	Thermal properties of milk	Spain	No	
UR-E26-(60)-9	Methods for purification of protein complexes	Sweden	Yes	
UR-E29-(60)-41	Studies on selected enzymes of milk	United Kingdom	Yes	1,2-C-2
UR-S3-(60)-10	Active site of trypsin	Brazil	Yes	1,2-C-1
E6 2-106(C)	Fraction of milk fat for specific food uses	Not yet determined	No	
E6 2-110(C)	Commercial scale development of low-fat Cheddar-type cheese	Not yet determined	No	
E6 2-112(C)	Fluid milk from nonfat dry milk and butteroil	Not yet determined	No	

* Discontinued during report year.
** Initiated during report year.

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
E6 5	Meat Utilization Investigations. Program Leadership			
E6 5-17 (Rev.)	Chemical, physical, and biological factors involved in the development of rancidity in fats, fatty tissues, and meats	Beltsville, Md.	Yes	3-B
E6 5-19 (Rev.)	Studies on the recovery and identification of substances responsible for flavor and aroma in meat	Wyndmoor, Pa.	Yes	3-D
E6 5-20 (Rev.)	Chemical reactions involved in meat-curing	Wyndmoor, Pa.	Yes	3-C
E6 5-21 (C)	A histochemical study of components of meat connective tissues and their relation to tenderness	Baton Rouge,La.	Yes	3-C
E6 5-22 (Rev.)	Studies of psychrophilic microorganisms	Beltsville,Md.	Yes	3-A
E6 5-23	Improving the flavor of cured meats through a study of the interrelationships of temperature, curing substances, microbial metabolism and mutation rates	Beltsville,Md.	Yes	3-A
E6 5-24 (Rev.)	Meat protein composition and distribution in relation to tenderness and juiciness	Wyndmoor, Pa.	Yes	3-C
E6 5-26 (C)	Fungi associated with meat processing and flavor development	Ames, Iowa	Yes	3-A
E6 5-27	Identifying substances in wood smoke that contribute to the flavor and aroma of meats	Wyndmoor,Pa.	Yes	3-D
E6 5-28	Development of new or improved meat processing methods and of new meat products	Wyndmoor,Pa.	Yes	3-C
E6 5-30 (C)	Development of new smoked meat products	East Lansing,Mich.	Yes	3-D
E6 5-31 (C)	New frozen meat products and their time-temperature relationships	Columbia, Mo.	Yes	3-B
E6 5-32 (Gr.)	The relationship of amounts and ratios of heme pigments to oxidative rancidity	Tallahasse,Fla.	Yes	3-B
E6 5-33**	The development of an accurate laboratory method of estimating the thermal history of meat products	Wyndmoor,Pa.	Yes	3-C
E6 5-34 (Gr.)**	A study of the nature and significance of non-carbonyl volatile compounds associated with rancidity in meats	New Brunswick, N. J.	Yes	3-B
UR-A6-(60)-4**	New semi-dehydrated fried meat products	Taiwan	Yes	3-C
UR-E8-(60)-14	Influence of fats on flavor and aroma of dry sausage	Finland	Yes	3-B
UR-E19-(60)-17**	The use of protozoa to detect harmful substances in meat	Netherlands	Yes	3-A
UR-E21 (60)-24	Antioxidant components of wood smoke used in meat-curing	Poland	Yes	3-D
UR-E29-(60)-15*	Enzymes attacking animal connective tissue	United Kingdom	Yes	3-C
UR-E29-(60)-70	Specific reducing systems in pork muscle	United Kingdom	Yes	3-C

* Discontinued during report year.
** Initiated during report year.

LINE PROJECT CHECK LIST -- REPORTING YEAR 64 to 65

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area of Subheading
E6 3	Animal Fats and Oils and Special Products Utilization Investigations. Program Leadership.			
E6 3-55 (Rev.)	Long-chain fat derivatives for polymer modification	Wyndmoor, Pa.	Yes	4-B-1
E6 3-58 (Rev.)	Soap-detergent combinations based on animal fats	Wyndmoor, Pa.	Yes	4-C-1
E6 3-61 (C)	Substituted vinyl monomers and polymers	Tucson, Arizona	Yes	4-B-1
E6 3-62	Synthetic lubricants from animal fats	Wyndmoor, Pa.	Yes	4-B-2
E6 3-63*	Organic-inorganic fat compounds for use in plastics	Wyndmoor, Pa.	No	
E6 3-64	Structure of components and derivatives of animal fats	Wyndmoor, Pa.	Yes	4-A-1
E6 3-65	Fractionation and analysis of lipids	Wyndmoor, Pa.	Yes	4-A-1
E6 3-66	Polymerizable amides from animal fats	Wyndmoor, Pa.	Yes	4-B-1
E6 3-67 (C)	Structural characteristics of organic peroxides	Pittsburgh, Pa.	Yes	4-D-2
E6 3-68	Autoxidation of fatty materials in emulsion	Wyndmoor, Pa.	Yes	4-A-2
E6 3-69 (C)(Rev.)	Spatial interrelations within triglyceride molecules	Villanova, Pa.	Yes	4-A-1
E6 3-70 (C)	X-ray investigations of a mixed triglyceride	Villanova, Pa.	Yes	4-A-1
E6 3-71 (C)	Interfacial adsorption characteristics of salts of alkyl esters of α-sulfo fatty acids as related to their wetting and detergent action	Bethlehem, Pa.	Yes	4-A-1
E6 3-72	Development of industrially useful chemicals by free radical addition products	Wyndmoor, Pa.	Yes	4-D-1
E6 3-73	Biodegradable detergents from animal fats	Wyndmoor, Pa.	Yes	4-C-1
E6 3-74 (Gr)	Synthesis of pure glycerides	Storrs, Conn.	No	
E6 3-75 (Gr)	Ozonization of animal fats	Austin, Minn.	No	
E6 3-76	New mathematical approaches to physical measurement	Wyndmoor, Pa.	Yes	4-A-1
E6 3-78 (C)**	High pressure hydrolysis of animal fats to alcohols without simultaneous chain saturation	Chicago, Ill.	No	
E6 3-80**	Synthesis of chemical intermediates by the introduction of reactive ester, sulfur, and oxygen functional groups into animal fats	Wyndmoor, Pa.	No	
E6 3-81 (Gr)**	Biological synthesis of unsaturated fatty acids	Not yet determined	No	
UR-E21-(40,60)-28**	Kinetics and thermodynamics of fat autoxidation	Gdansk, Poland	No	
UR-E25-(60)-22	Cocoa butter substitutes from animal fats	Madrid, Spain	Yes	4-A-1
UR-A7-(60)-72	Preparation and properties of long chain sulfated monoglycerides	Bombay, India	No	
UR-E9-(60)-79	Hydroxylated fatty derivatives	Marseille, France	Yes	4-B-1
UR-E9-(60)-88**	Autoxidation of fat at low temperatures	Paris, France	No	

* Discontinued during report period.
**Initiated during report period.

LINE PROJECT CHECK LIST -- REPORTING YEAR 64 to 65

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Summary of Progress	Incl. in Area and Subheading
E6 4	Hides, Skins and Leather Utilization Investigations. Program Leadership			
E6 4-32	Composition and properties of animal protein residues	Wyndmoor, Pa.	Yes	5-D-1
E6 4-34	Processes for making commercial quality leather from enzyme-unhaired hides	Wyndmoor, Pa.	Yes	5-C-1
E6 4-35	New tanning processes for producing leathers of superior durability	Wyndmoor, Pa.	Yes	5-C-2
E6 4-36	Effect of electrolytes and lipid components on hide properties	Wyndmoor, Pa.	Yes	5-A-3,4
E6 4-37(C)	Noncollagenous proteins of cattlehides	Cincinnati,Ohio	Yes	5-A-4
E6 4-38 (C)(Rev.) **	Preparation and properties of dispersed collagen sols	Kansas City, Mo.	No	
E6 4-39	Microscopic investigation of skin and leather structure	Wyndmoor, Pa.	Yes	5-A-2
E6 4-40 (C)	Abnormalities of leather characterized by a depleted mush texture	Cincinnati,Ohio	Yes	5-C-3
E6 4-41	Physical properties of collagen and leathers	Wyndmoor, Pa.	Yes	5-A-1
E6 4-42	Addition of new reactive sites to hide proteins	Wyndmoor, Pa.	Yes	5-B-2
E6 4-43	Chemical modification of hides with aldehydes in combination with phenols, amides, hydrazides	Wyndmoor, Pa.	Yes	5-B-3
E6 4-44	Chemical modification of animal hides with cyclic urea derivatives such as urons and triazones	Wyndmoor, Pa.	Yes	5-B-3
E6 4-45 (Gr)	Physical properties of collagen	Evanston, Ill.	Yes	5-A-3
E6 4-46 (C)**	Physical, chemical and engineering aspects of drying leather	Not yet determined	No	
E6 4-47 (C)**	New products through dispersion and reconstitution of collagen fibers	Not yet determined	No	
E6 4-48**	Regenerated collagen products for use in food products	Wyndmoor, Pa.	No	
E6 4-49**	Dehydration of animal hides and skins	Wyndmoor, Pa.	No.	
E6 4-50***	New tanning processes from application of new chemical modification agents	Wyndmoor, Pa.	Yes	5-C-2
UR-A7-(60)-17	Polyphenolic tanning compounds	Madras, India	Yes	5-A-1
UR-A7-(60)-18	Relation of hide quality to tanning rate	Madras, India	Yes	5-C-5
UR-E8 (60)-3	Fractionation of gelatin and collagen	Turku, Finland	Yes	5-A-1
UR-E15-(60)-5****	Microbial damage to exported U. S. hides	Naples, Italy	Yes	5-C-6
UR-A7-(60)-43	Hydrothermal shrinkage of collagen and leather	Madras, India	Yes	5-A-1
UR-E19-(60)-13	Kinetics of chrome tanning	Waalwijk,Holland	Yes	5-C-4
UR-E29-(60)-67	Chemically reactive compounds for improving leather stability	Surrey, England	Yes	5-B-3
UR-A7-80**	Radioactive tracer study of mineral tanning	Madras, India	No	
UR-A7-81**	The comfort properties of shoe leathers	Madras, India	No	
UR-A7-92**	Rapid tannage of sole leather	Madras, India	No	

* Superseded by E6 4-50.
** Initiated during report period.
*** Supersedes E6 4-35.
**** Discontinued during report period.

LINE PROJECT CHECK LIST -- REPORTING YEAR 64 to 65

Work & Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area of Subheading
E3 6	Potato and Other Vegetable Utilizations - Eastern Region. Program Leadership	Wyndmoor, Pa.		
E3 6-42*	Nitrogenous constituents as quality factors in potato processing	Wyndmoor, Pa.	Yes	6-A-1
E3 6-44	Basic composition studies on the lipid fraction of potatoes	Wyndmoor, Pa.	Yes	6-A-3
E3 6-45	Basic studies on the formation and identity of the after-cooking discoloration pigment	Wyndmoor, Pa.	Yes	6-A-5
E3 6-46	Color and texture of frozen French fries	Wyndmoor, Pa.	Yes	6-B-1
E3 6-47	Effect of varietal, cultural and other source factors on the quality of processed potato products	E. Grand Forks, Minnesota	Yes	6-B-3
E3 6-48**	Pilot plant investigations on methods for producing dehydrated potato pieces capable of rapid rehydration by modification of internal structure and components related to texture	Wyndmoor, Pa.	Yes	6-C-1
E3 6-49**	Basic studies on proteins of potatoes	Wyndmoor, Pa.	Yes	6-A-4
E3 6-50**	Pigments formed during frying of chips	Wyndmoor, Pa.	Yes	6-A-2
E3 6-41	Development of new types of dehydrated vegetables through modification of internal structure	Wyndmoor, Pa.	Yes	7-A-1; 7-B-1

* Discontinued during report year.
** Initiated during report year.

LINE PROJECT CHECK LIST -- REPORTING YEAR 64 to 65

Work & Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
E3 3	Apples and Other Fruit Utilization Investigations - Eastern Region. Program Leadership	Wyndmoor, Pa.		
E3 3-30*	Development of improved apple cider	Wyndmoor, Pa.	Yes	8-B-4
E3 3-32	Rapidly-reconstitutable dried fruit products	Wyndmoor, Pa.	Yes	8-B-1,8-B-2,8-B-3,8-C-1, 8-C-2
E3 3-33 (C)	Relation of physical and chemical properties to processing characteristics of eastern peaches	New Brunswick, N. J.	Yes	8-C-4
E3 3-34	Improvement of processed cherries through studies on composition and post-harvest treatments	Wyndmoor, Pa.	Yes	8-A-1,8-C-3
E3 3-35 (C)	Relation of apple cell wall constituents to textural quality of processed products	College Park,Md.	Yes	8-A-2
E3 3-36 (Gr)**	Radioactive tracer studies of cherry well wall constituents	Philadelphia,Pa.	No	
E3 3-37**	Development of improved cider and cider products	Wyndmoor, Pa.	No	

* Discontinued during report period.
** Initiated during report period.

LINE PROJECT CHECK LIST -- REPORTING YEAR 64 to 65

Work & Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Summary of Progress	Incl. in Area and Subheading
E5 3	Tobacco Investigations. Program Leadership	Wyndmoor, Pa.		
E5 3-5	Acid and bases in cigar smoke	Wyndmoor, Pa.	Yes	9-A-2
E5 3-6	Composition of cigarette smoke	Wyndmoor, Pa.	Yes	9-A-1, 9D
E5 3-7	Composition of oxidation products	Wyndmoor, Pa.	Yes	9-B-1, 9C
E5 3-8 (C)(Rev.)	Investigations of neutral resins	Durham, N. C.	No	9-B-2
E5 3-9 (C)*	Modifications of cigarette burn temperature	Linwood, Pa.		
E5 3-10*	Evaluation of cigarette modifiers	Lexington, Ky.	No	
E5 3-11 (C)*	Improved method for polynuclear hydrocarbons in smoke	Lexington, Ky.	No	
E5 3-12 (C)*	Pyrolysis products of amino acids	Lexington, Ky.	No	
E5 3-13 (C)*	Heterocyclic bases of cigarette smoke	Lexington, Ky.	No	
E5 3-14 (C)*	Improved biological assaying	Lexington, Ky.	No	
E5 3-15 (C)*	Routine biological assaying	Lexington, Ky.	No	
E 5 5	New and Replacement Crops Utilization Investigations. Program Leadership	Wyndmoor, Pa.		
E5 5-39**	New crop seed epoxy-containing oils	Wyndmoor, Pa.	Yes	12-A

* Initiated during report period.
** Discontinued during report year.

Work & Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area and Subheading
E5 1	Sugar and Sirups Investigations. Program Leadership	Wyndmoor, Pa.		
E5 1-67(C)*	Improvement in processing and quality by study of factors affecting sugar sand formation	Wooster, Ohio	No	
E5 1-68*	Isolation, characterization and properties of honey enzymes	Wyndmoor, Pa.	Yes	11-A
E5 1-74	Chemical compounds responsible for maple flavors; precursors of maple flavor	Wyndmoor, Pa.	Yes	10-A-1
E5 1-76	Improvement of the quality of maple products through a study of the fermentation-induced biochemical reactions involved in the formation of maple color and flavor	Wyndmoor, Pa.	Yes	10-B-1,2
E5 1-78(C)	Improvement of the maple industry through development of methods for prolonged storage of sap; control of microbial fermentation of sap before and after delivery to storage tanks	Bainbridge, N.Y.	Yes	10-C-1,2

* Discontinued during report year.